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Angiotensin II Stimulates Interleukin-6 Release From Cultured Mouse Mesangial Cells[1]

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ABSTRACT

Interleukin-6 (IL-6) is a multifunctional cytokine exerting a wide variety of biologic responses, including cell proliferation. Recently, IL-6 has been known to play a role in the pathogenesis of mesangial proliferative glomerulonephritis. IL-6 is now recognized as an autocrine growth factor for glomerular mesangial cells, and various inflammatory mediators have been shown to promote IL-6 release from mesangial cells. However, little is known about the noninflammatory stimuli of IL-6 release from mesangial cells. In this study, it was hypothesized that angiotensin II (AngII) is one of the noninflammatory mediators of IL-6 release in mesangial cells, and the effects of AngII on IL-6 release and mRNA expression in cultured mouse mesangial cells (CMMC) were investigated. It was demonstrated that AngII (10^{-7} M or higher) caused IL-6 release and mRNA accumulation in CMMC. IL-6 release was detected at 4 h and reached a plateau at 8 h after the addition of AngII, whereas IL-6 mRNA expression peaked at 4 h. The effects of AngII on IL-6 release and gene expression were completely blocked by the AngII receptor type 1 (AT1 receptor) antagonist CV-11974. AngII and IL-6 were both shown to stimulate DNA synthesis in CMMC, and the blockade of IL-6 signaling with anti-IL-6 receptor antibody abolished the enhanced DNA synthesis induced by AngII. These results raise a possibility that the growth-promoting effect of AngII on mesangial cells is at least partially mediated by IL-6 released from mesangial cells.

Key Words: *Vasoactive peptide, cytokine, glomerulonephritis, autocrine growth factor, AT1 receptor antagonist*

Mesangial hypercellularity and matrix expansion are hallmarks of glomerular diseases and occur commonly in all chronic glomerular diseases at their advanced stage. The involvement of several mediators has been suggested in the pathogenesis of these morphologic and functional changes of glomeruli. Among the mediators that have attracted attention are vasoactive peptides, cytokines, and polypeptide growth factors (1,2). Interleukin-6 (IL-6) has been suggested to play a role in proliferative glomerular disease. The expression of IL-6 in glomeruli was demonstrated by several authors in renal biopsy specimens from patients with proliferative glomerulonephritis (3–5). IL-6 has been demonstrated to promote mesangial cell proliferation in rat (3,6) and mouse (7) *in vitro*. IL-6 also induces fibronectin gene expression in cultured mesangial cells obtained from human fetus (8). Transgenic mice harboring the IL-6 gene, which overexpresses IL-6, presented a glomerular lesion characterized by hypercellularity and matrix expansion in the mesangial region (9). IL-6 production in cultured mesangial cells has been shown in rat (3,6) and mouse (7). Several factors stimulate IL-6 release from cultured mesangial cells; these factors include platelet-derived growth factor, fetal calf serum (FCS), lipopolysaccharide, IL-1, and tumor necrosis factor-alpha (3,5–7,10). Most of these factors are related to the inflammatory reaction in glomeruli, suggesting the importance of immunologic insults as a trigger for the initiation of the disease. However, in the majority of patients—those who are in the process of progression to glomerulosclerosis from these initial insult—there is little ongoing immunologic injury (1,2). The existence of IL-6 in intrinsic glomerular cells, even in the chronic phase of glomerulonephritis, when the acute inflammatory process has terminated, necessitates the involvement of other stimulator(s) of this cytokine release than the inflammatory mediators listed above. We hypothesized that angiotensin II (AngII) is one of the noninflammatory stimulators of IL-6 release from mesangial cells in the chronic phase of glomerulonephritis, because AngII has been suggested to be involved in the progression of the glomerular lesion, on the basis of the observation of renoprotective effects of angiotensin-converting enzyme inhibitor in both clinical and experimental settings (11,12). In this article, we demonstrated that AngII causes gene expression and the release of IL-6 in cultured mouse mesangial cells (CMMC). AngII stimulated DNA synthesis in CMMC, and the stimulated DNA synthesis was abolished by the concomitant blockade of IL-6 action with the anti-IL-6 receptor antibody, suggesting the partic-

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ipation of IL-6 in AngII-induced CMMC growth in an autocrine manner.

METHODS

Materials

The sources for some of the materials used were as follow: AngII was from Peptide Inc. (Osaka, Japan). The AngII specific receptor antagonist CV-11974 (the active form of TCV-116) (13) was a gift from Takeda Chemical Industries, Ltd (Osaka, Japan). Human recombinant IL-6, IL-6-dependent murine hybridoma MH60.BSF2 cells were kindly provided by T. Matsuda and T. Hirano (Division of Molecular Oncology, Biomedical Research Center, Osaka University School of Medicine). 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H tetrazolium bromide (MTT) was from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). *O*-Tetradecanoylphorbol 13-acetate (TPA) was from Sigma (St. Louis, MO). Anti-α-smooth muscle actin monoclonal antibody was obtained from Immunotech S.A. (France). Antivimentin and antidesmin monoclonal antibodies were purchased from Lipshaw (MI). [α-^{32}P]dATP (3,000 Ci/mmol) was purchased from Du Pont, NEN Research Products (Boston, MA).

CMMC

In this study, we chose the C57BL/6 mouse as a source of mesangial cells because of the two reasons described below. (*1*) In the IL-6 transgenic mouse study (9), this strain of mouse was used as a host animal for IL-6 gene transfer. (2) Anti-IL-6 receptor antibody was available in mouse, but not in rat. Male C57BL/6 mice weighing approximately 15 to 17 g were euthanized under ether anesthesia. After kidneys were removed, renal cortices were dissected from the kidney, minced, and passed through three steel meshes of different sizes (120, 75, and 53 μm). The glomeruli retained on the last mesh were suspended in RPMI 1640 medium (Nissui, Japan) supplemented with 20% fetal calf serum (FCS) (Cell Culture Laboratories, Cleveland, OH), 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin (Sigma). Isolated glomeruli were incubated in plastic dishes at 37°C in 5% CO_2. Outgrowth of mesangial cells was observed 3 to 4 days after seeding. Spindle-shaped mesangial cells usually approached confluence within 3 wk. Mesangial cells were identified morphologically and by positive staining for α-smooth muscle actin, desmin, and vimentin (14). CMMC were also tested for AngII binding, and specific AngII binding to CMMC was observed (data not shown). All experiments were performed with passage 2 or 3 CMMC. Cells were maintained in serum-free RPMI 1640 medium containing 0.5% bovine serum albumin (BSA) for 2 days before experiments, except for the proliferation assay.

IL-6 Assay

IL-6 activity in the culture supernatant was measured with MH60.BSF2 cells as described by Matsuda *et al.* (15) with some modification. Briefly, cells were cultured for 54 h in a 96-well plate (Costar, Cambridge, MA) with samples or human recombinant IL-6 standard. The proliferation of cells was determined by use of the colorimetric MTT assay (16). Ten microliters of MTT (5 mg/mL in phosphate-buffered saline; PBS) was added to each well during the last 6-h incubation; then, the cells were dissolved in 0.04 N HCl/2-propanol. The uptaken MTT dye was measured colorimetrically at 540 nm on a microplate reader (Titertek Multiskan CMMCC/340, EFLAB, Finland). AngII and AngII receptor antagonist had no direct effect on MH60.BSF2 cell proliferation at the concentrations used in this study.

Anti-IL-6 Receptor Antibody

Polyclonal antibody to mouse IL-6 receptor was prepared and kindly provided by Dr. T. Matsuda and Dr. T. Hirano (Osaka University School of Medicine). New Zealand White rabbits were immunized with recombinant murine IL-6 receptor as has been reported (17), and antiserum against IL-6 receptor was obtained. Antibody was partially purified from the serum by Protein A-Sepharose (Pharmacia, Piscataway, NJ) column chromatography. The blocking activity of the antibody toward the signaling via the IL-6 receptor was tested with the IL-6-dependent hybridoma cell MH60.BSF2. MH60.BSF2 cells were pretreated with control antibody or anti-IL-6 receptor antibody for 1 h before the addition of recombinant IL-6 (final, 1 U/mL); then the proliferation of the MH60.BSF2 cells was determined by the MTT assay already described. Anti-IL-6 receptor antibody inhibited the MH60.BSF2 cell proliferation induced by IL-6 in a concentration-dependent manner (64, 80, and 95% inhibition at 2.5, 5, and 10 μg/mL antibody, respectively), whereas control antibody did not affect the proliferation of MH60.BSF2 cells, even at 10 μg/mL.

RNA Extraction and Northern Analysis

Total cellular RNA was prepared by the acid guanidinium thiocyanate-phenol-chloroform extraction procedure (18). Cells were rinsed with ice-cold PBS twice, and then 1 mL of denaturing solution (4 M guanidine thiocyanate, 25 mM sodium citrate [pH 7.0] 0.5% sarcosyl, 0.1 M 2-mercaptoethanol) was added. Then, cells were scraped from the dish with a rubber policeman (Costar), transferred to a tube, and passed through a needle with a syringe five times to shear genomic DNA. Sequentially, 0.1 mL of sodium acetate (pH 4.0), 1 mL of phenol (water saturated), and 0.2 mL of chloroform were added to the cell lysate. The final suspension was shaken vigorously for 10 s and placed on ice for 15 min. Samples were centrifuged at 10,000 *g* for 20 min at 4°C; then, the aqueous phase was transferred to a fresh tube, mixed with 2.5 mL of ethanol, and placed at −20°C for at least 1 h. The tubes were centrifuged at 10,000 *g* for 20 min, and the resultant pellet was dissolved in water; then, phenol-chloroform extraction followed by ethanol precipitation was performed. The final RNA pellet was dissolved in an adequate volume of water, and RNA was quantitated by optical density at 260 nm.

Standard manipulation of DNA and RNA was done according to Sambrook *et al.* (18). Thirty micrograms of RNA was fractionated on 0.8% agarose-formaldehyde gel, transferred to nylon membrane filters (Gene Screen; Du Pont-NEN), and cross-linked by ultraviolet wave irradiation. The *Xho*I fragment of mouse IL-6 cDNA (about 600 base pairs) was used as a probe for IL-6 (kind gift from Dr. T. Hirano, Osaka University School of Medicine). The rat rRNA gene (19) was kindly provided by the Japanese Cancer Research Resources Bank. The *Eco*RI/*Bam*HI fragment (about 1 kb) of the coding region of the rRNA gene was used as a probe for the detection of rRNA. ^{32}P labeling of the DNA was done by the random priming method (20). Prehybridization of the membranes was done for 2 h at 42°C in a buffer containing 5× saline-sodium phosphate-EDTA buffer (SSPE) (1× SSPE = 150 mM NaCl, 11 mM NaH_2PO_4, 2 mM EDTA), 10× Denhardt's (100× Denhardt's = 2% Ficoll, 2% polyvinylpyrroridone, 2% BSA), 0.5% sodium dodecyl sulfate (SDS), 100 μg/mL denatured

salmon sperm DNA, and 50% (vol/vol) formamide. The membranes were hybridized with 10^6 cpm/mL IL-6 probe in hybridization buffer (same as prehybridization buffer) for 24 h at 42°C. After hybridization, filters were washed twice for 15 min in 0.1× saline sodium citrate buffer (SSC) (20× SSC = 3 M NaCl, 0.3 M sodium citrate; pH 7.0) with 0.1% SDS at 60°C. The membranes were then autoradiographed with intensifying screens (Du Pont, Wilmington, DE) at −70°C for 3 to 5 days. Blots were then washed for 10 min in 0.1% SDS at 80°C to remove the hybridized IL-6 cDNA. Then, the filters were rehybridized with the rRNA probe.

Measurement of DNA Synthesis

Subconfluent CMMC grown on 24-well plates were used. Cells were maintained in RPMI 1640 medium containing 0.5% FCS, insulin (5 μg/mL), and 20 mM *N*-hydroxyethylpiperazine-*N*′-2-ethanesulforic acid (pH 7.4) for 48 h; then AngII (10^{-7} M) or IL-6 (500 U/mL) was added in combination with anti-mouse IL-6 receptor antibody or control immunoglobulin G. Twenty-four hours after the addition of the reagents, cells were pulsed with [^{3}H]thymidine (0.5 μCi/well) for 24 h. Then, cells were rinsed with PBS three times; the incorporated [^{3}H]thymidine was fixed by washing them twice with ice-cold trichloroacetic acid (5%) and was extracted with 1 N NaOH over 24 h, followed by neutralization with HCl. An aliquot was measured for radioactivity in a liquid scintillation spectrometer (Aloka, Tokyo, Japan).

Statistics

The significance of difference was determined by *t* test when there were only two groups in an experiment and by Dunnett's test when there were three or more groups in an experiment. *P* values < 0.05 were considered to be significant.

RESULTS

Effect of IL-6 on MTT Uptake of MH60.BSF2 and Demonstration of IL-6 Bioactivity in the Culture Supernatant of CMMC Stimulated by AngII

The effect of IL-6 on the MTT uptake of MH60.BSF2, a murine IL-6-dependent hybridoma clone, was examined, and a dose-response curve for the IL-6 bioassay was established. As shown in Figure 1, the MTT dye uptake of MH60.BSF2 cells was log linear against a log concentration of IL-6 over a wide range (0.004 to 1 U/mL). AngII (10^{-6} M), CV-11974 (10^{-5} M), and TPA (1 μg/mL) did not affect the MTT uptake of MH60.BSF2 cells. This result excludes the possibility that AngII itself promotes the growth of MH60.BSF2 directly and leads to a false-positive for IL-6 bioactivity. The standard curve for the IL-6 bioassay with MH60.BSF2 cells was prepared for every set of bioassay. Next, we tested if IL-6 bioactivity was detected in the culture supernatant of CMMC treated with AngII. CMMC were cultured in RPMI 1640 medium containing 0.5% BSA for 48 h; then, the cells were stimulated by AngII (10^{-7} M) in otherwise the same medium for 8 h. Culture supernatant was collected and was used for the IL-6 bioassay. To verify that the growth promotion of MH60.BSF2 by the culture supernatant of CMMC was mediated by IL-6 activity, we used anti-

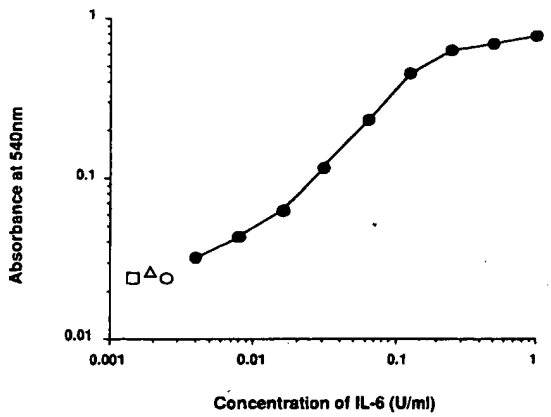

Figure 1. Effect of IL-6 on the MTT uptake of MH60.BSF2 cells. Ten thousand MH60.BSF2 cells were cultured with various concentrations of IL-6 (closed circles), 10^{-6} M AngII (open square), 10^{-6} M CV-11974 (open triangle), or 1 μg/ml TPA (open circle) for 48 h; then MTT dye was added to the culture for 6 h. Standard error was within 5% of the mean of triplicates.

mouse IL-6 receptor antibody to block the IL-6 receptor pathway. The culture supernatant of AngII-stimulated CMMC induced MH60. BSF2 proliferation, which was inhibited by anti-mouse IL-6 receptor antibody (Figure 2). Thus, IL-6 bioactivity was demonstrated in the supernatant of AngII-stimulated CMMC.

Time Course of IL-6 Release From CMMC on AngII Stimulation

Next, we tested the time course of IL-6 release after the addition of AngII. Cells were made quiescent in RPMI 1640 medium containing 0.5% BSA for 48 h

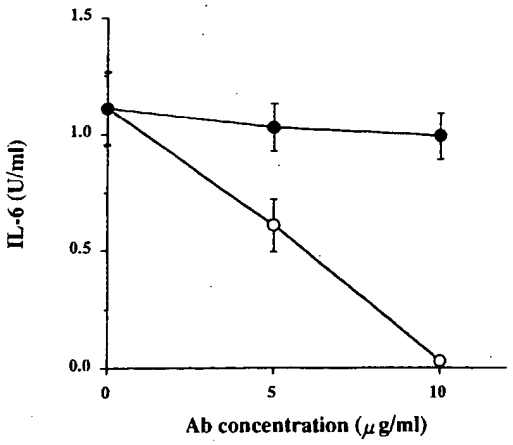

Figure 2. Demonstration of IL-6 bioactivity in the culture supernatant of CMMC. The IL-6 bioassay of culture supernatant from CMMC stimulated by AngII was performed in the presence of anti-murine IL-6 receptor antibody (Ab) (open circles) or isotype-matched control antibody (closed circles). CMMC were stimulated by AngII (10^{-7} M) for 8 h. Results are expressed as mean ± SE (*N* = 3).

before the addition of AngII. At Time 0, AngII (10^{-7} M) was added; then, the IL-6 bioactivity in the culture supernatant was assayed after 4, 8, 10, and 24 h of incubation. At Time 0, little IL-6 bioactivity was detected in the culture supernatant; then, it increased with time until it reached plateau at 8 h. There was no further increase in IL-6 bioactivity in the culture supernatant past 8 h until 24 h (Figure 3).

Dose-Dependent Effect of AngII on IL-6 Release From CMMC

We next tested the effect on IL-6 release from CMMC of different concentrations of AngII ranging from 10^{-6} M to 10^{-11} M (Figure 4). Cells were cultured in serum-free RPMI 1640 containing 0.5% BSA for 48 h and then were stimulated by AngII for 8 h. A significant increase in IL-6 bioactivity was observed when 10^{-7} M or higher concentration of AngII was used.

Effect of AngII Specific Antagonist on IL-6 Release From CMMC

At least two types of AngII receptor, AT1 and AT2, are known, and AT1 was demonstrated to be a dominant receptor in cultured mesangial cells (21). Therefore, we tested the effect of the AT1 antagonist CV-11974 on IL-6 release from CMMC stimulated by AngII. CV-11974 (10^{-5} and 10^{-6} M) completely blocked the IL-6 release induced by 10^{-7} M AngII. CV-11974 alone had no effect on IL-6 release from CMMC (Figure 5). This result indicates that AngII-dependent IL-6 release from CMMC is mediated by the AT1 receptor.

IL-6 Gene Expression in CMMC

Northern blot analysis was performed to test if the IL-6 gene expression is induced in CMMC by AngII. CMMC were maintained in serum-free RPMI 1640 containing 0.5% BSA for 48 h; then, AngII was added to the medium. CMMC were incubated in the presence of 10^{-7} M AngII for 0, 4, 8, 12, 16, and 24 h (Figure 6). The effect of the AT1 receptor antagonist (CV-11974) was also tested. CMMC expressed no IL-6 mRNA at Time 0, but hybridized bands were seen past 4 h until 24 h after the addition of AngII. Signal intensity showed a peak at 4 h, and then it decreased with time. The effect of AngII on IL-6 mRNA expression was almost completely abolished by CV-11974 (10^{-6} M). The dose-dependent effect of AngII on the IL-6 mRNA in CMMC was also studied (Figure 7). CMMC were

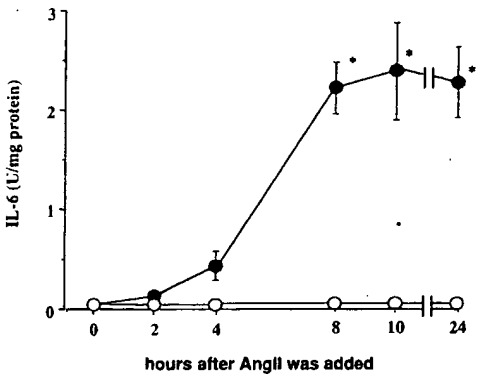

Figure 3. Time course of IL-6 release from 10^{-7} M AngII-stimulated CMMC. CMMC were maintained in the serum-fr medium containing 0.5% BSA for 48 h. At Time 0, 10^{-7} M AngII (closed circles) or vehicle (open circles) was added to th m dium. At th indicated time, the sup rnatant was coll cted. R sults ar xpr ssed as mean ± SE (N = 3). *P < 0.01 *versus* control.

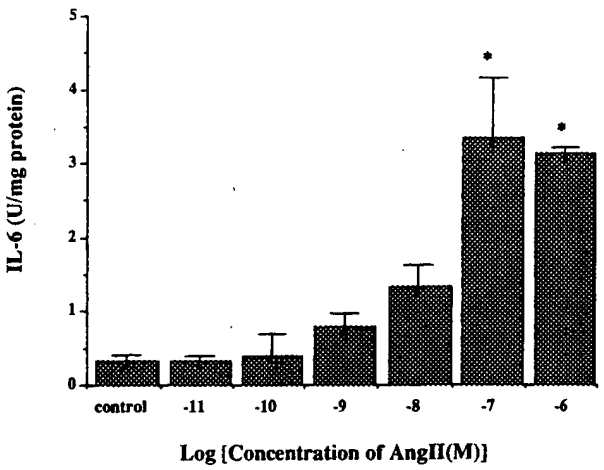

Figure 4. Effect of various concentrations of AngII on IL-6 release from CMMC. CMMC were cultured in the serum-free RPMI 1640 containing 0.5% BSA for 48 h before the experiment. Then, cells were incubated in the presence of different concentrations, from 10^{-11} to 10^{-6} M, of AngII for 8 h. Results are expressed as mean ± SE of triplicates. *P < 0.01 *versus* control.

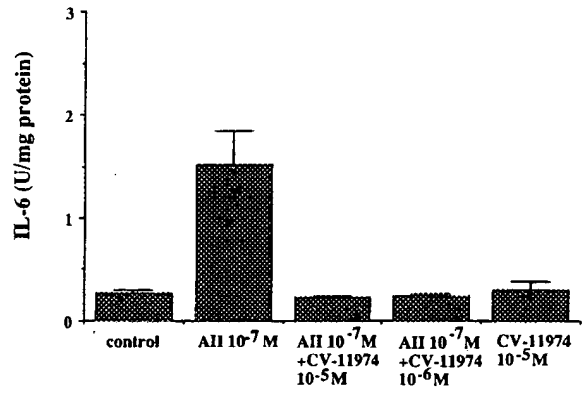

Figure 5. Inhibitory effect of CV-11974, an AT1 receptor antagonist, on AngII-induced IL-6 release from CMMC. CMMC were maintained in the serum-free RPMI 1640 containing 0.5% BSA for 48 h before the experiment. Then, cells were treated for 8 h with AngII (10^{-7} M), AngII plus CV-11974 (10^{-5} M), AngII plus CV-11974 (10^{-6} M), or CV-11974 (10^{-5} M) alone. Results are expressed as mean ± SE (N = 3). AII, AngII.

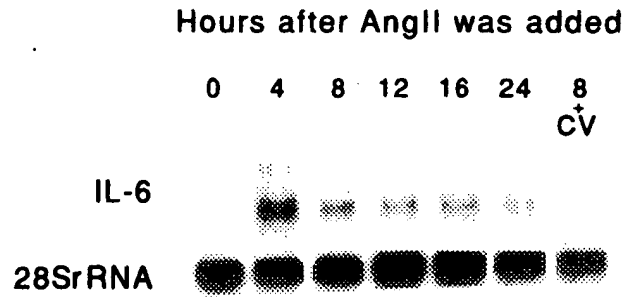

Figure 6. Time course of the expression of IL-6 mRNA induced by AngII. CMMC were maintained in serum-free RPMI 1640 containing 0.5% BSA for 48 h before the addition of AngII (10^{-7} M). After 4, 8, 12, 16, and 24 h of incubation with AngII, total RNA was isolated. The effect of CV-11974 (10^{-6} M), an AngII receptor (AT1) antagonist, was also tested at 8 h. CV-11974 was added in combination with AngII. Thirty micrograms of total RNA from each point was loaded onto an agarose-formaldehyde gel. CV, CV-11974; 28SrRNA, 28S rRNA.

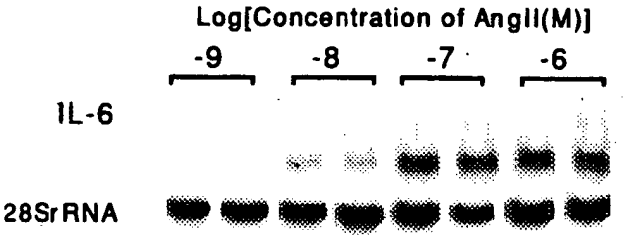

Figure 7. Effect of various concentrations of AngII on IL-6 mRNA expression in CMMC. CMMC were cultured in serum-free RPMI 1640 containing 0.5% BSA for 48 h before the addition of AngII. After 4 h of incubation with various concentrations of AngII (10^{-9} to 10^{-6} M), total RNA was isolated. Thirty micrograms of total RNA from each culture plate was used for one lane. 28SrRNA, 28S rRNA.

maintained in the serum-free medium (containing 0.5% BSA) for 48 h; then AngII (10^{-9}, 10^{-8}, 10^{-7}, and 10^{-6} M) was added to the medium. After 4 h of incubation, total RNA was isolated from CMMC and northern blotting was performed. A faint band was seen at 10^{-8} M, and substantial signals were detected at 10^{-7} and 10^{-6} M AngII. Autoradiographs of rRNA were shown to verify the equality in the amount of RNA loaded per lane.

DNA Synthesis of CMMC in Response to AngII and IL-6

We investigated the role of AngII-induced IL-6 secretion in the growth response of CMMC to AngII. CMMC were maintained in the RPMI 1640 containing 0.5% FCS and insulin (5 μg/mL) for 48 h before the experiment. Then, cells were stimulated for 24 h in the medium containing the various reagents indicated. A significant increase in [^{3}H]thymidine incorporation per well was observed after AngII (10^{-7} M) treatment (Figure 8A), and the coadministration of anti-IL-6

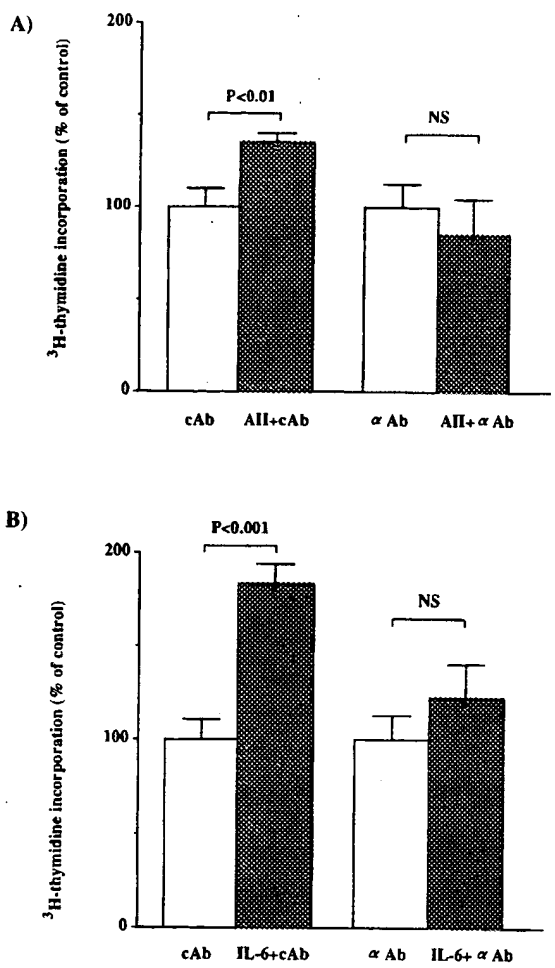

Figure 8. [^{3}H]thymidine uptake of CMMC in response to AngII and IL-6. Cells were maintained in RPMI 1640 containing 0.5% FCS and insulin (5 μg/mL) in 24-well plates for 48 h before th experiment. Twenty-four hours after the addition of AII (10^{-7} M) or IL-6 (500 U/mL) plus either IL-6 receptor antibody (10 μg/mL) or control immunoglobulin G (10 μg/mL), cells were pulsed with [^{3}H]thymidine for 24 h. (A) Effect of AngII. (B) Effect of IL-6. Results are expressed as relative values of corresponding control (mean ± SE; N = 4). AII, AngII; cAb, control antibody; αAb, anti-IL-6 receptor antibody. Control values of [^{3}H]thymidine uptake for cAb and αAb were: cAb; 146,272 cpm/well, αAb; 164,299 cpm/well.

receptor antibody abolished the enhanced DNA synthesis in response to AngII. IL-6 (500 U/mL) also significantly increased the incorporation of [^{3}H]thymidine in CMMC, and the coadministration of anti-IL-6 receptor antibody reduced the IL-6-induced DNA synthesis to the control level (Figure 8B). The anti-IL-6 receptor antibody itself had no toxic effect on CMMC, because similar [^{3}H]thymidine uptake was seen between control antibody-treated and anti-IL-6 receptor antibody-treated CMMC. Further, there was no difference in the viability of the cells between control antibody-treated and anti-IL-6 receptor antibody-treated

CMMC after 48 h of incubation with antibodies, as judged by the number of Trypan blue–stained cells (data not shown). We also tested whether this increase in DNA synthesis caused by AngII or IL-6 treatment was accompanied by a change in cell number by MTT assay under the identical condition for the [^{3}H]thymidine incorporation assay. No significant difference in MTT uptake was seen among control, AngII-, or IL-6-treated CMMC (control, 0.856 ± 0.070; AngII, 0.829 ± 0.103; IL-6, 0.874 ± 0.059; mean ± SD of the O.D. values from five wells).

DISCUSSION

In this study, we found that AngII is capable of stimulating IL-6 release from CMMC. In rat adrenal zona glomerulosa cells, AngII was shown to increase IL-6 release at 10 nM or greater concentration after 4.5 to 5 h of incubation (22). In CMMC, we found that 100 nM or higher Ang II caused significant release of IL-6. Although both AngII and IL-6 have been shown to be involved in the pathogenesis of glomerular lesions, no report has ever suggested the interaction between AngII and IL-6, and this is a unique demonstration of "vasoactive peptide-cytokine interaction" in mesangial cells. This action of AngII was shown to be mediated by AT1 receptor in CMMC AngII caused IL-6 mRNA accumulation in CMMC. The time course for IL-6 gene expression showed a peak at 4 h, when IL-6 bioactivity in the medium started to increase significantly. The promoter region of the human IL-6 gene has an NF-κB-binding site, and this binding site was demonstrated to be responsible for gene activation by a variety of IL-6 inducers, including TPA (23). Various agents that are known to activate protein kinase C (PKC) in intact cells rapidly induce the DNA-binding activity of NF-κB. The binding of NF-κB to the NF-κB-binding site of the IL-6 gene is an important step for the efficient induction of IL-6 gene transcription. In the human fibroblast cell line FS-4, PKC activation was shown to increase the expression of the IL-6 gene (24). PKC activation is a part of AngII signaling in the mesangial cells (25,26), and we found that the PKC activator (TPA) caused IL-6 release from CMMC (data not shown). In rat adrenal glomerulosa cells, the PKC activator increased IL-6 release (21). Other vasoactive peptides like arginine vasopressin and endothelin-1 are also known to cause polyphosphoinositides turnover and subsequent PKC activation in mesangial cells (25). We also found that arginine vasopressin and endothelin-1 are both capable of inducing IL-6 release from CMMC in a fashion similar to AngII (27). Thus, it seems likely that AngII stimulates IL-6 gene transcription via PKC activation.

The next important question is the function of the released IL-6 from CMMC after the stimulation of AngII. IL-6 has been thought to be an autocrine growth factor of cultured mesangial cells, because exogenously added IL-6 promotes mesangial cell proliferation *in vitro*. By using the IL-6 receptor-specific antibody to block the effect of IL-6 through its receptor, we demonstrated that the released IL-6 may play a role in the growth response of CMMC to AngII. The amount of IL-6 released from CMMC was 5 to 10 U/6-cm plate, resulting in the concentration of approximately 3 U/mL in the medium. This concentration of IL-6 is apparently less than the effective IL-6 concentration on the mesangial cell proliferation, which is at least 100 U/mL according to previous works (3,6,7). The difference could be attributed to the autocrine and/or paracrine nature of secreted IL-6; the local concentration of IL-6 near its receptor could be much higher than the observed concentration, which depends on the dilution factor determined by the amount of medium supplied per plate.

We also confirmed the growth-promoting effects of AngII (28–30) and IL-6 (3,6,7) on cultured mesangial cells described already. In this study, we performed a DNA synthesis assay in the presence of 0.5% serum and insulin (5 μg/mL) and observed a significant increase in DNA synthesis after AngII stimulation. No stimulation of DNA synthesis was seen in serum or insulin-free conditions (data not shown). Interestingly, Ikeda *et al.* reported that IL-6 inhibited the DNA synthesis of rat mesangial cells in the presence of 0.5% FCS, but no inhibition of DNA synthesis by IL-6 was noted in the presence of 5 or 20% FCS (31). The reason for the discrepancy between their observation and ours is not clear. It could be attributed to the difference of the species or the passage number of the cells used (they used 6 to 10 passages of rat mesangial cells) or the experimental condition (for example, they did not add insulin to the growth assay culture). Although significant increases in DNA synthesis were observed in CMMC after stimulation by either AngII or IL-6, the increase in the cell number determined by the MTT assay did not reach a statistical significance in response to either of them. The increase in DNA synthesis was relatively small, and the difference in cell number was not detected by the MTT assay, which is less sensitive compared with the [^{3}H]thymidine incorporation assay. Alternatively, increased DNA synthesis may occur both in hyperplastic and in hypertrophic growth with polyploidity (32). In vascular smooth muscle cells, AngII is known to stimulate hypertrophic growth, and the growth is modulated by the balance of multiple autocrine growth factors such as platelet-derived growth factor, basic fibroblast growth factor, and transforming growth factor-β, which are expressed by the vascular smooth muscle cells after AngII stimulation (33). In light of the analogy of the mesangial cells to the vascular smooth muscle cells, similar modulation of mesangial cell growth by multiple growth factors including IL-6 may occur; however, this possibility needs further investigation to be established.

AngII has been thought to deteriorate glomerular lesions mainly by its effect on intraglomerular hemodynamics; AngII causes glomerular hypertension via systemic hypertension and direct vasoconstrictive ac-

tion on the efferent arteriole of glomeruli (12). In recent years, in addition to the hemodynamic effect of AngII, direct actions of AngII on glomerular cells, including a growth-promoting effect, have attracted much attention in the context of the pathogenesis of AngII in glomerulosclerosis (12,25). Our finding that AngII causes IL-6 release from mesangial cells offers a new aspect of the direct action of AngII on mesangial cells, and the possible involvement of secreted IL-6 in the growth-promoting effect of AngII raises a new insight in the pathogenesis of glomerulosclerosis from a viewpoint of "vasoactive peptide-cytokine interaction."

The results of this study illustrate the interaction of AngII and IL-6 in CMMC. AngII was shown to stimulate IL-6 release and mRNA accumulation in CMMC via the AT1 receptor. Both AngII and IL-6 had growth-promoting effects on CMMC, and the coadministration of anti-IL-6 receptor antibody decreased the AngII-induced increase in DNA synthesis to the control level, demonstrating the possible involvement of IL-6 as a growth factor in AngII-activated CMMC. Our results described here raise a possibility that the AngII-dependent progression of the glomerular lesion is at least partially mediated by IL-6 produced from mesangial cells.

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IL-6 functions in cynomolgus monkeys blocked by a humanized antibody to human IL-6 receptor

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Abstract

A humanized antibody to the human interleukin-6 receptor (IL-6R), hPM-1, blocked the interleukin-6 (IL-6) functions in normal cynomolgus monkey lymphocytes in vitro. The binding activity of hPM-1 to non-human primate IL-6R was examined in peripheral blood lymphocytes by flow cytometry. PM-1 recognized the IL-6R on T lymphocytes of cynomolgus and rhesus monkeys, but did not on those of marmosets. The homology between human IL-6R and its cynomolgus monkey counterpart was 97.3% in the extracellular domain of the amino acid sequence, as determined by DNA sequencing of the PCR product from peripheral blood mononuclear cells. PM-1 inhibited two functional parameters in vitro in cynomolgus monkeys: (1), T-cell proliferation stimulated by phytohemaglutinin and human IL-6; (2), Immunoglobulin G-production evoked by *Staphylococcus aureus* Cowan-I- and human IL-6-stimulated B lymphocytes.

These data show that hPM-1 binds to and functionally blocks the cynomolgus monkey IL-6 receptors.

Key words: IL-6; Humanized; PM-1; Monkey; DNA; Flow cytometry

Interleukin-6 (IL-6) is a multifunctional cytokine, regulating immune responses, nerve cells, myeloma cells, acute phase reactions and hematopoiesis (Kishimoto,1989; Satoh et al., 1998; Hirono *et al., 1990*; Klein et al., 1990; Winton et al., 1994). Abnormal expressions of the IL-6 gene leads to many diseases such as autoimmune diseases and multiple myeloma. A murine monoclonal anti-IL-6 antibody has been reported to inhibit the proliferation of myeloma cells in human patients (Klein et al., 1991; Legouffe et al., 1994;). However, murine antibody was immunogenic and elicited the production of antibodies against itself (Human anti-murine antibody; HAMA) which resulted in the decreased serum concentration of this therapeutic agent. In order to avoid the

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production of HAMA, humanized antibodies have been constructed (Brown et al., 1991; Hakimi et al., 1991; Delmonico et al., 1993). Recently, a new construct, hPM-1, a humanized antibody to human IL-6 receptor (IL-6R) was found to be equivalent to the original murine monoclonal antibody (mPM-1), in terms of antigen binding and in vitro growth inhibition of multiple myeloma cell lines (Hirata et al., 1989; Sato et al., 1993; Suzuki et al., 1992). In order to determine its preclinical effects on various IL-6 functions, we first selected compatible species in which IL-6R is recognized by hPM-1. As the homology between the human IL-6R and its mouse counterpart was as low as 48% in terms of amino acid sequences (Yamazaki et al., 1988; Sugita et al., 1990; Baumann et al., 1990;) PM-1 does not bind to mouse IL-6R. Therefore, we examined the effects of hPM-1 on the IL-6 functions using peripheral blood cells of primates. Since human IL-6 has been reported to elevate the serum levels of acute phase proteins and circulating platelet counts in these primates (Mayer et al., 1991; Asano et al., 1990; Stahl et al., 1991), a high degree of homology between human and non-human primate IL-6R was expected.

This report describes the homology of IL-6R among primates and the blockage of IL-6 functions by hPM-1 in the cynomolgus monkey.

1. Experimental procedures

1.1. T-lymphocyte preparation

Heparinized peripheral blood was obtained from a healthy human volunteer (after informed consent was obtained), from cynomolgus monkeys (*Macaca fascicularis*), rhesus monkeys (*Macaca mulatta*) and marmosets (*Callithrix jacchus*). The animals used in these experiments were treated in accordance with Chugai Pharmaceutical's ethical guidelines which meet the generally accepted international criteria for humane treatment. Mononuclear (MN) cells were prepared by density gradient centrifugation using Ficoll-paque (Pharmacia-LKB, Uppsala, Sweden). Red blood cells were lysed by incubating MN cells with 0.75% ammonium chloride, 17 mM Tris-HCl buffer (pH 7.65) at 25°C for 10 min. Non-adherent cells were separated from adherent cells after incubation in petri dishes (Code No. Falcon-3003, Becton Dickinson and Company [Becton D], Lincoln Park, NJ, U.S.A.) for 1 h in 5% CO_2/air at 37°C. Finally, non-adherent MN cells were applied to Cellect T-cell kit column (Funakoshi, Tokyo, Japan) to collect T lymphocytes which passed through. The purity was examined by flow cytometry (CD4, CD8, and CD19) as described later. The viability of all cell fractions was checked by the exclusion of trypan blue staining.

1.2. Flow cytometry

IL-6R expression on human MN cells and T lymphocytes was analysed by two-color flow cytometry. Aliquots of cells were incubated sequentially with murine PM-1 (mPM-1), phycoerythrin-labeled (PE-) anti-mouse immunoglobulin G (IgG) (Tago Inc., Burlingame, CA, U.S.A.) and fluorescein-coupled (FL-) monoclonal antibodies (MoAbs) against T cells and B cells; i.e. anti-CD4 (Mouse IgG_1, Nichirei, Tokyo, Japan), anti-CD8 (Mouse IgG_2a, Nichirei, Japan) and anti-CD19 (Mouse IgG_1, Becton D). When hPM-1 was used as the first antibody, FL-goat anti-human IgG (Organon Teknika Corp., Durham, NC, U.S.A.) was used as the secondary antibody and PE-

MoAbs against CD4 and CD8 (Becton D) were used to form the tertiary antibody complex. In order to examine the competitive inhibition between human IL-6 and PM-1, MN cells were pre-incubated with human IL-6 for 15 min and then incubated with PM-1. Stained cells were resuspended in phosphate-buffered saline (PBS⁻) containing 2% foetal bovine serum (Xavier Investments, Wacol, Australia) and 0.1% sodium azide and analysed using FACScan and FACStation software (Becton D). The following were used as control IgGs; FL-mouse IgG_1 (Becton D) for FL-anti-CD4 and FL-anti-CD19, FL-mouse IgG_2a (Becton D) for FL-anti-CD8, purified human IgG (Organon Teknika) for hPM-1 and MOPC31C (purified IgG1) for mPM-1. Antibodies against such human antigens as CDs and IgGs were also used to detect their respective monkey counterparts; we could not purchase antibodies against monkey antigens due to a Washington treaty to protect rare animals and plants.

1.3. Partial cloning of cynomolgus monkey IL-6R by PCR-DNA sequencing

We designed PCR primers based on the human IL-6R sequence to amplify the cDNA of cynomolgus monkey IL-6R and determined the nucleotide sequence of the extra-cellular domain, except for 14 amino acids at the N-terminus. The mRNA was isolated from cynomolgus monkey MN cells using the Micro-FastTrack mRNA isolation kit (Invitrogen, San Diego, CA, U.S.A.) for synthesizing double-stranded cDNA using the TimeSaver cDNA synthesis kit (Pharmacia-LKB). PCR primers were designed using Oligo software (National Biosciences, Plymouth, MN, U.S.A.) and were synthesized using DNA synthesizer 394 (Perkin Elmer-Cetus, Norwalk, CT, U.S.A.). Their sequences were as follows: 5′-GCGCAGGAGGTGGCAAGAGG-3′ (Position 516–535), 5′-TCCAGCAACCAGGAATGTGG-3′ (Position 1531–1550), 5′-CAGCAACGTT-GTTTGTGAGT-3′ (Position 818–837; an internal primer). The PCR was carried out for 35 cycles of 94°C (30 s), 54°C (60 s) and 72°C (90 s) using the GeneAmp PCR reagent kit (Takara, Kyoto, Japan) and DNA Thermal cycler 480 (Perkin Elmer-Cetus). The supposed PCR product was isolated by low melting-point agarose-gel electrophoresis and was subcloned into pUC18 vector using the SureClone ligation kit (Pharmacia-LKB). The DNA sequence was determined by the Sequenase DNA sequencing kit (Toyobo, Osaka, Japan) using [(α-^{32}P]dCTP with universal primers M4, RV and the internal primer.

1.4. Proliferation (T lymphocytes) and IgG production (B lymphocytes) assays

The proliferation of T lymphocytes was assessed as follows. T lymphocytes depleted of B lymphocytes were cultured at 1×10^5/well in RPMI-1640 medium (Gibco, Grand Island, NY, U.S.A.) supplemented with 1% foetal bovine serum (Xavier Investments), 1 μg/ml phytohemaglutinin (PHA) (Sigma, St Louis, MO, U.S.A.) and 1 ng/ml human IL-6 with/without hPM-1 in 5% CO_2/air at 37°C for 4 days. T lymphocytes were pulsed with 1 μCi/ml ^{3}H-thymidine (5Ci/mmol, Amersham, Japan, Tokyo, Japan) for 4 h and the incorporated radioactivity was measured using the liquid scintillation analyser Betaplate (Pharmacia-LKB).

The IgG production by B lymphocytes was assessed as follows: non-adherent MN cells were cultured in RPMI-1640 medium (Gibco) supplemented with 10% foetal bovine serum (Xavier Investments), 0.01% *Staphylococcus aureus* Cowan 1 (SAC, Calbiochem, La Jolla, CA, U.S.A.) for 2 days. These stimulated cells were washed and then cultured with/without mPM-1 and human

IL-6 in 5% CO_2/air at 37 °C for 5 days. The amount of cynomolgus monkey IgG produced in the culture supernatant was determined by the enzyme-linked immunosorbent assay (ELISA) described below.

1.5. ELISA

ELISA was performed according to the previously reported method (Goldsmith *et al.*, 1981).

Immunoplate-II (Nunc, Denmark) was coated with purified goat IgG against human IgG (Organon Teknika Corp.) in 0.1 M sodium bicarbonate buffer (pH 9.5) containing 0.02% sodium azide at 4 °C overnight to detect cynomolgus monkey IgG. Wells were washed three times with PBS containing 0.05% tween 20 (Rinse buffer) and 0.05 M Tris-HCl buffer (pH 8.1) containing 1% bovine serum albumin, 1 mM $MgCl_2$, 0.15 M NaCl, 0.05% tween 20 and 0.02% sodium azide was added to block non-specific binding. After discarding the blocking buffer, samples were incubated at 25 °C for 1 h. The plate was washed and then alkaline phosphatase-conjugated goat anti-human IgG (Organon Teknika) was added. After 1 h, the microplate was washed and developed with the substrate Sigma 104 (Sigma) in 0.05 M sodium bicarbonate buffer containing 0.01 M $MgCl_2$ (pH 9.8). The absorbance at 405 nm was determined using Multiskan MCC/340 (Eflab, Helsinki, Finland). The IgG concentration was calculated from a calibration curve, with standard cynomolgus monkey IgG purified in-house using a protein-A column.

2. Materials

2.1. Antibodies

PM-1, a murine MoAb to human IL-6R, was produced and characterized as previously described (Hirata et al., 1989) and its humanized antibody (hPM-1) was constructed by grafting complementarity-determining regions (CDRs) as reported (Sato et al., 1993).

3. Results

3.1. Flow cytometry

The cross-reactivity of PM-1 among IL-6Rs of primates was examined by two-color flow cytometry using peripheral blood T lymphocytes. Such lymphocyte subsets as $CD4^+$, $CD8^+$ and $CD19^+$ cells were identified by staining with a single MoAb (data not shown). First, human T lymphocytes were used as the positive control in comparison with the cynomolgus monkey counterpart (Fig. 1). Murine PM-1 could bind to human $CD4^+$ lymphocytes and not the $CD8^+$ cells. Since it is known that binding of PM-1 to IL-6R can be inhibited by human IL-6, we examined the competitive inhibition between PM-1 and human IL-6 using human T lymphocytes. The binding of mPM-1 to human $CD4^+$ cells was inhibited competitively and also dose-dependently by human IL-6 added in advance. These data suggested that the binding of mPM-1 to human $CD4^+$ cells occurred via the IL-6R. With regard to other species, T lymphocytes of cynomolgus and rhesus monkeys were also stained by mPM-1 and the competitive inhibition between mPM-1

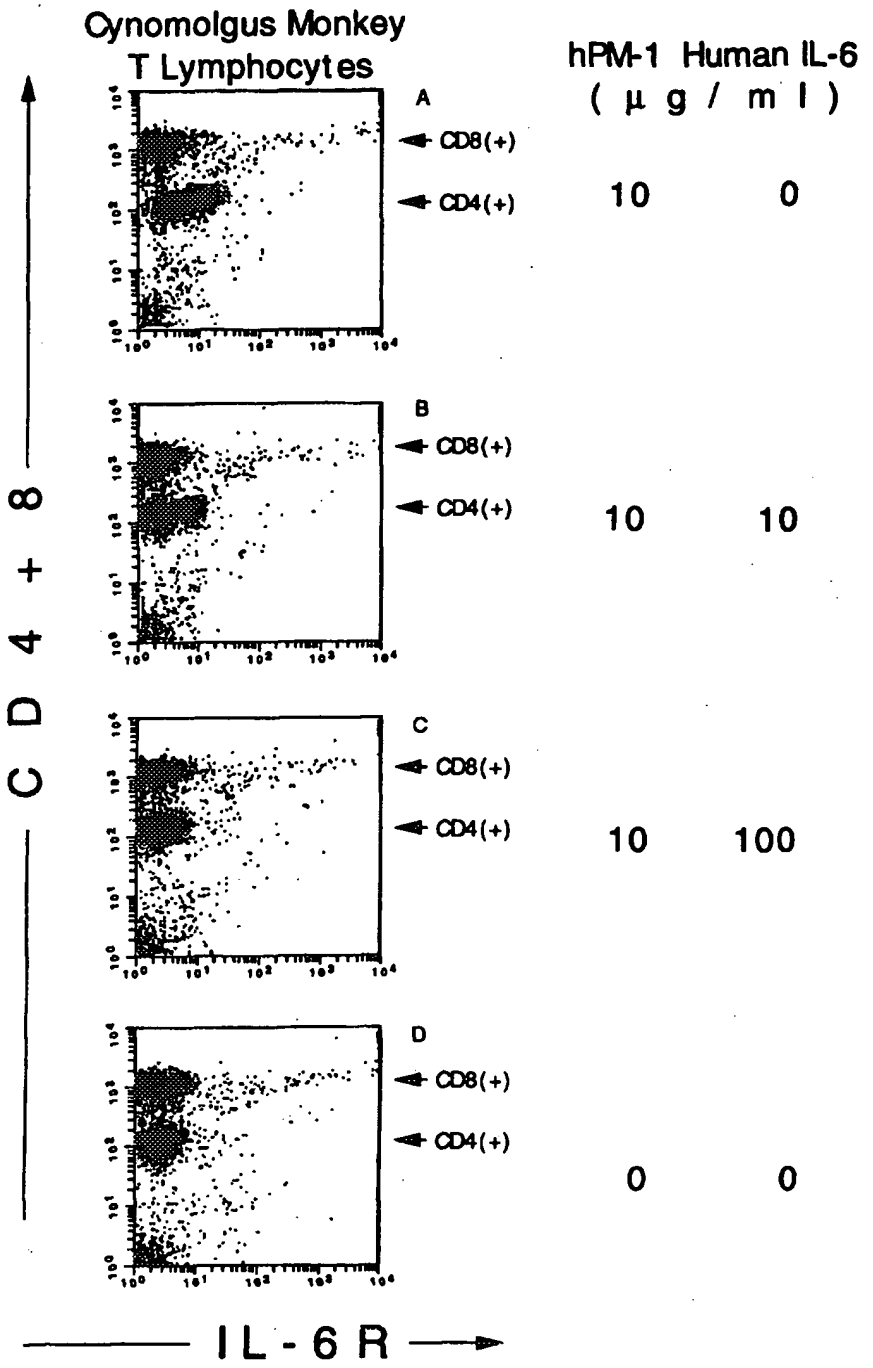

Fig. 1. Flow cytometric two-color analysis of IL-6R on T lymphocytes. T lymphocytes (prepared as described in Materials and methods) were incubated sequentially with mPM-1, PE-anti-mouse IgG and mixtures of FL-MoAbs against CD4 and CD8. To examine the competitive inhibition between human IL-6 and mPM-1, T lymphocytes were *pre-incubated* with human IL-6 before *incubation with mPM-1 (B,C).*

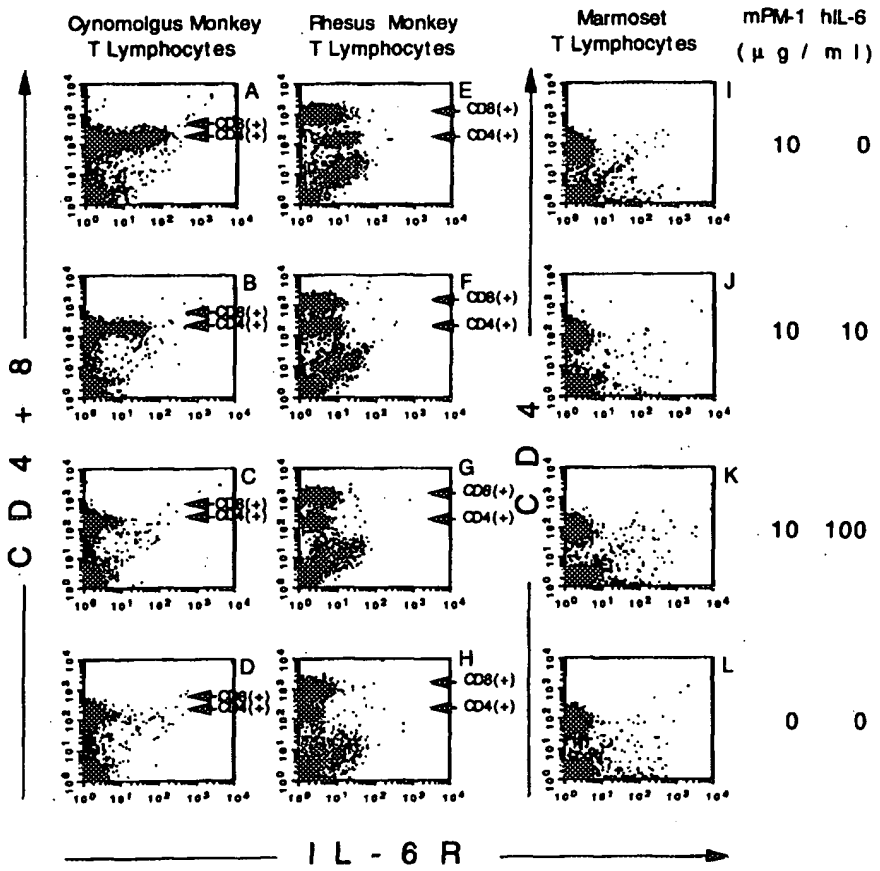

Fig. 2. Flow cytometric two-color analysis of IL-6R on non-human primate T lymphocytes. T lymphocytes (prepared as described in Materials and methods) were incubated sequentially with mPM-1, PE-anti-mouse IgG, and mixtures of FL-MoAbs against CD4 and CD8 (A–H) or against CD4 alone (I–L) To examine the competitive inhibition between human IL-6 and mPM-1, T lymphocytes were pre-incubated with human IL-6 before incubation with mPM-1 (B,C,F,G,J,K).

and human IL-6 was observed in the same manner (Fig. 2). Conversely, those of the marmoset were not stained by mPM-1. Here we used a MoAb against CD4 alone, because marmoset CD8 was not detected clearly by anti-human CD8 antibody which demonstrated low cross-reactivity between human and cynomolgus monkey in our previous studies. Furthermore, hPM-1 bound cynomolgus monkey T lymphocytes in the same fashion as mPM-1 (Fig. 3).

3.2. Partial sequence of cynomolgus IL-6R determined by PCR cloning

Figure 4 shows the partial sequence of human IL-6R and its cynomolgus monkey counterpart in terms of nucleotides (above the line) and amino acids (below the line) and indicates sequence differences. Twenty-five substitutions of the nucleotide sequence were observed, whereas only nine

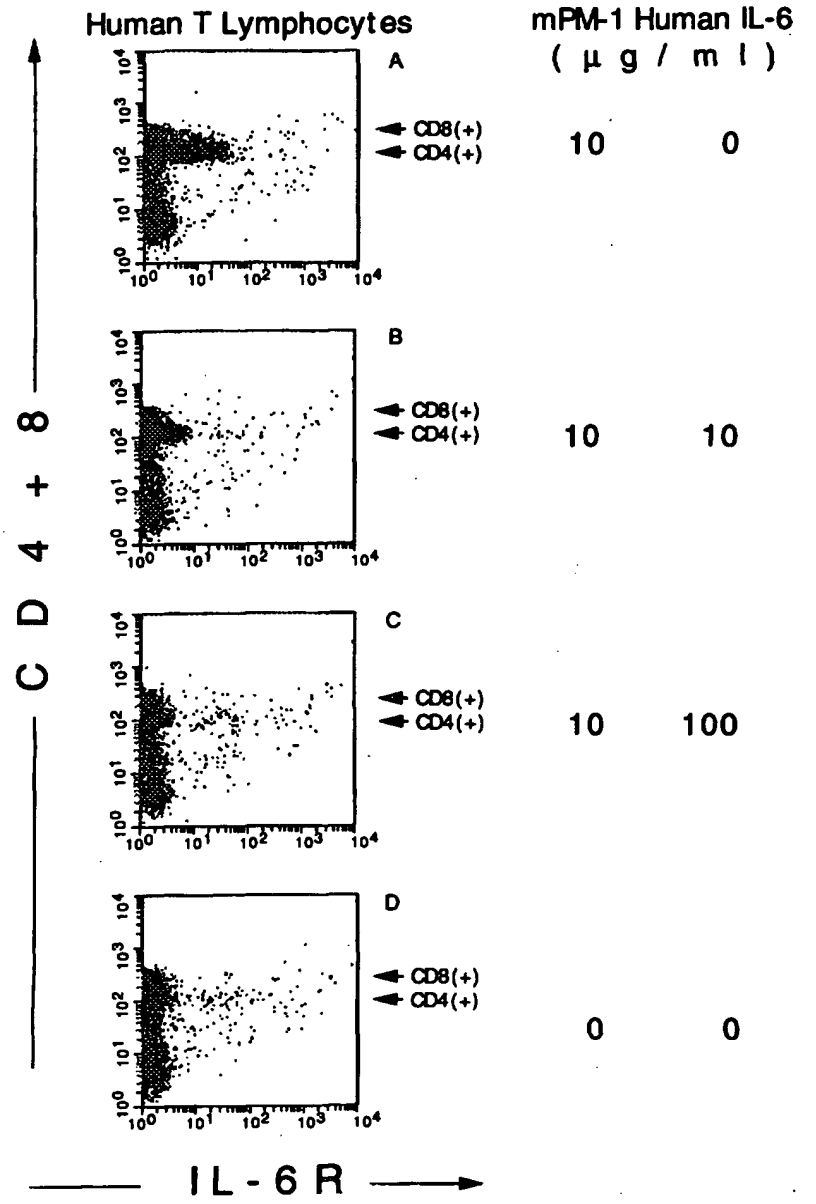

Fig. 3. Flow cytometric two-color analysis of IL-6R on cynomolgus monkey T lymphocytes with hPM-1. T lymphocytes (prepared as described in Materials and methods) were incubated sequentially with hPM-1, FL-anti-human IgG and mixtures of PE-MoAbs against CD4 and CD8. To examine the competitive inhibition between human IL-6 and hPM-1, T lymphocytes were pre-incubated with human IL-6 before incubation with hPM-1 (B,C).

existed in the amino acid sequence in cynomolgus IL-6R; V50G, A67V, P71L, M77V, L142P, F202L, M331T, L334P, F345S. The *IL-6R homology* between human and cynomolgus monkey was as high as 97.5% (970/995) with respect to the nucleotide sequence and 97.3% (322/331) with

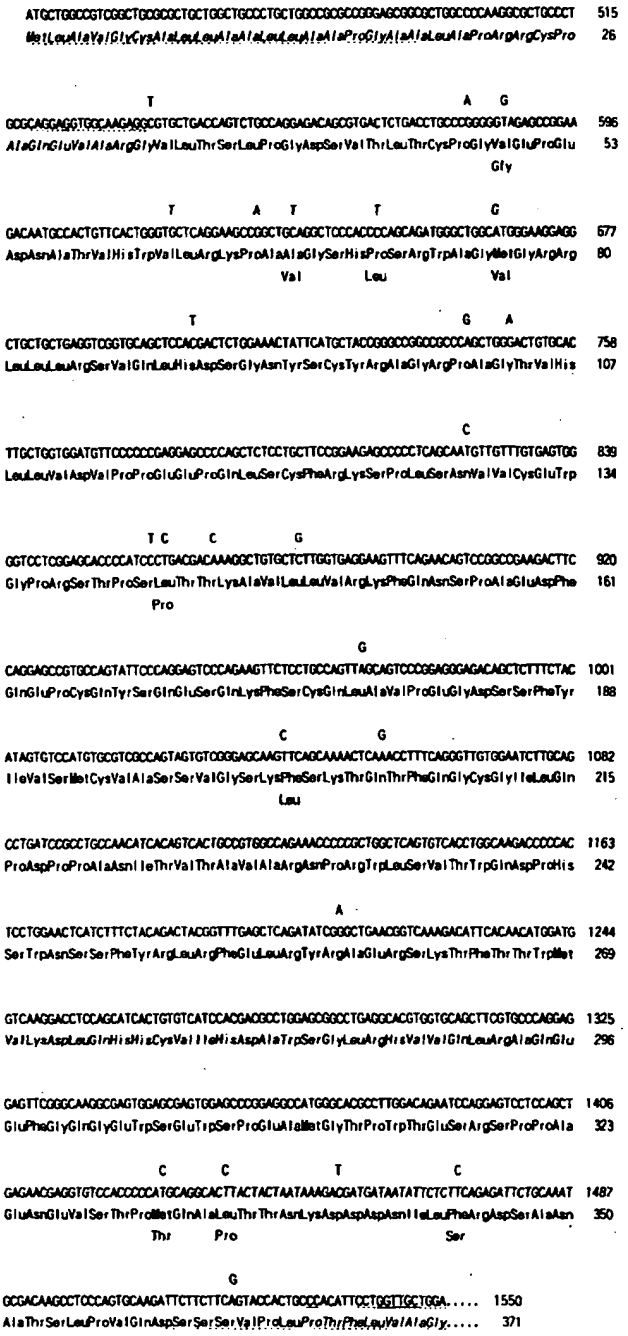

Fig. 4. Partial sequences of the cynomolgus IL-6R gene and amino acids. Base-pairs differing from the corresponding ones of the human IL-6R sequence are shown above the line and different amino acids are shown below the line. The unbroken line shows the primer sequences; the broken line shows signal sequences and trans-membrane regions, and italics show sections which could not be determined in this work.

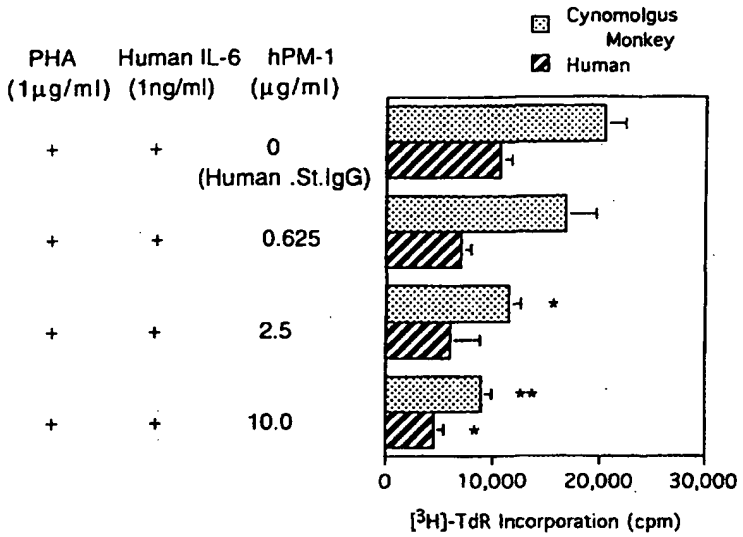

Fig. 5. Inhibited proliferation of PHA-stimulated T lymphocytes by hPM-1. T lymphocytes were cultured with PHA (1 μg/ml) and human IL-6 (1 ng/ml) with/without hPM-1 for 4 days. They were then pulsed with ^{3}H-thymidine for 4 h and the incorporated radioactivity was measured. Data were represented as mean ± standard error (SE) Negative control values with PHA alone without IL-6 were 4866 ± 934 and 14,224 ± 471 by human and cynomolgus monkey T lymphocytes respectively. Significant differences from the human standard IgG group were examined using Student's *t*-test ($n = 3$): * $P < 0.05$, ** $P < 0.01$.

respect to the amino acid sequence. We also tried to clone marmoset IL-6R by PCR, but failed to obtain an amplified product with these primers (data not shown).

3.3. *Blocked IL-6 functions*

The proliferation of cynomolgus monkey T lymphocytes stimulated by PHA and human IL-6 was inhibited by hPM-1 (Fig. 5). Although T lymphocytes can be stimulated by PHA alone, PHA and IL-6 had additive stimulatory effects. Humanized PM-1 inhibited the additive proliferation completely at a concentration of 10 μg/ml. IgG production can also be stimulated additively with SAC and IL-6 (Fig. 6). Murine PM-1 blocked the additive IgG production by cynomolgus monkey MN cells completely at a concentration of 10 μg/ml. In these experiments, we used murine PM-1 in order to avoid the cross-reactivity of alkaline phosphatase-labeled secondary antibody against the added humanized PM-1 and the IgG produced by the cynomolgus monkey.

4. Discussion

In this study we demonstrated the homology of IL-6R among primates and the effective blockage of IL-6R functions by hPM-1 in cynomolgus monkey lymphocytes. Fortunately, PCR primers designed for the human IL-6R could be used to amplify the corresponding gene in cynomolgus

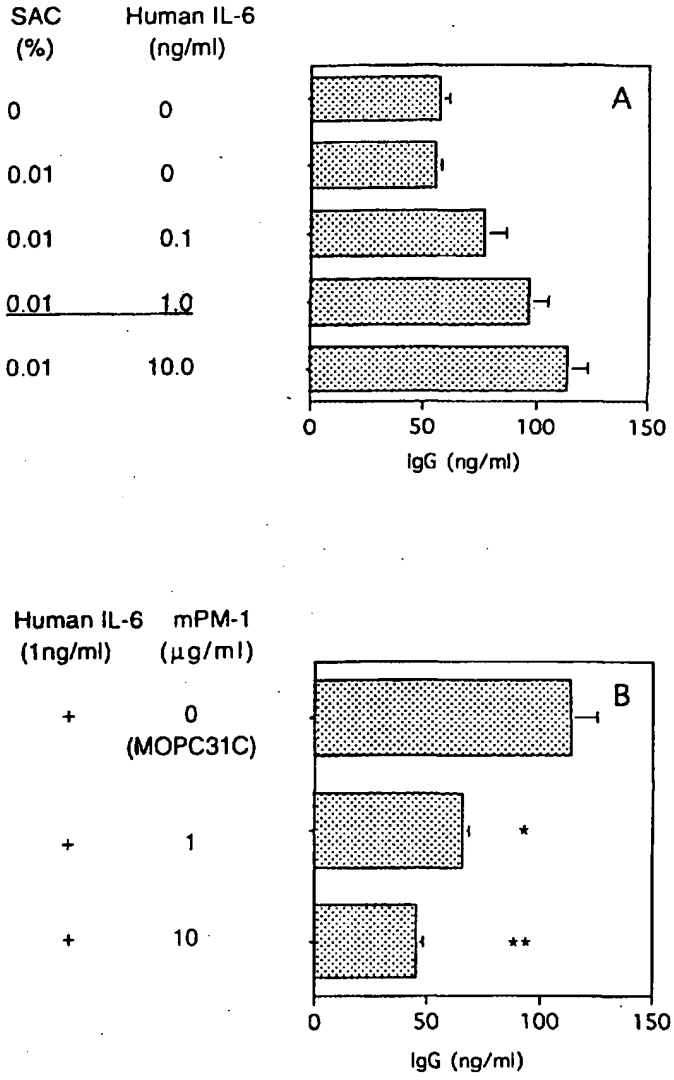

Fig. 6. IgG production by cynomolgus monkey MN cells blocked by mPM- 1. A: Non-adherent MN cells were cultured in 0.01% SAC and various concentrations of human IL-6. The concentration of human IL-6 used for this assay was determined as 1 ng/ml. B: Non-adherent MN cells were cultured in SAC (0.01%) and human IL-6 (1 ng/ml) with/without mPM-1, where MOPC (10 μg/ml) was added as a control IgG instead of mPM-1. The amount of cynomolgus monkey IgG produced in the culture supernatant was measured by ELISA. Data were represented as mean ± standard error (SE) The negative control value with SAC alone without IL-6 was 55.2 ± 2.8. Significant differences from the MOPC group were examined using Student's *t*-test ($n = 6$): * $P < 0.05$; ** $P < 0.01$.

monkeys, because the homology was greater than 97%. The marmoset IL-6R gene, however, could not be amplified with these primers. The "degenerated PCR" technique may be necessary and useful for amplifying the IL-6R gene in other strains and species when homology of IL-6R is

lower. The IL-6R of the cynomolgus monkey had no substitutions of the amino acid residues responsible for binding IL-6 and transducing the IL-6 signal via gp130, as predicted from a site-directed mutagenesis study of hIL-6 (Yawata et al., 1993). Of nine amino acid substitutions, seven were located in the Ig-like domain (20P-110V) and the transmembrane region (T316-D358) which were not critical for binding IL-6 or mediating the IL-6 signal. Two other substitutions at the positions of L142P and F202L, which belong to the cytokine receptor family domain (D111-W315), are known not to be critical for either of these functions. Therefore, hPM-1 binds to the cynomolgus IL-6R and blocks its function. Although there were 14 amino acids that we could not identify at the N-terminus, the partial sequence determined in this study was sufficient to predict the functions of cynomolgus IL-6R, as most of the critical sequence was included. It is clear that the PCR-DNA sequencing technique is simple and is useful for studying the species differences with many ligands and receptors, where homology is expected to be high to some extent.

We also determined the IL-6R strain differences among primates by flow cytometry. Since PM-1 and hIL-6 are known to compete (Hirata et al., 1989), we confirmed that the binding of PM-1 to monkey lymphocytes was due to IL-6R, and was not due to non-specific binding. However, we could not determine the exact homology numerically by flow cytometry, as by the PCR-DNA sequencing method, because the amount of bound antibody depends on the types of both epitopes and recognition antibodies in flow cytometry. In contrast with several reports (Taga et al., 1987; Wognum et al., 1993; Yoshizaki et al., 1990), $CD8^+$ cells were apparently not stained by PM-1 in these lymphocyte subsets. IL-6R expression levels on $CD8^+$ cells are known to be so much lower than on $CD4^+$ cells that they would not be detectable. Detection would therefore require a more sensitive method such as the avidin-biotin complex technique.

It is known that hPM-1 has similar binding capacities to the IL-6Rs of several human myeloma cell lines as mPM-1, and therefore exhibits the same anti-tumour activity (Sato et al., 1993; Suzuki et al., 1992). This study showed that hPM-1 not only bound to the IL-6R but also inhibited the functions of cynomolgus monkey lymphocytes.

The homology of IL-6R and cross-reactivity of hPM-1 to primate IL-6R revealed in this study will allow us to examine both the pharmacological efficacy and safety of hPM-1 in these non-human primates.

Acknowledgements

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Development of a Human Interleukin-6 Receptor Antagonist*

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Neutralizing monoclonal antibodies specific for human interleukin-6 (IL-6) bind two distinct sites on the IL-6 protein (sites I and II). Their interference with IL-6 receptor binding suggested that site I is a receptor-binding site of IL-6, whereas site II is important for signal transduction. Mutagenesis of site II could therefore result in the isolation of IL-6 receptor antagonists. To test this hypothesis, a panel of IL-6 mutant proteins was constructed that did not bind to a site II-specific monoclonal antibody. One such site II mutant protein (with double substitution of Gln-160 with Glu and Thr-163 with Pro) was found to be an antagonist of human IL-6. It was inactive on human CESS cells, weakly active on human HepG2 cells, but active on mouse B9 cells. It could specifically antagonize the activity of wild-type IL-6 on CESS and HepG2 cells. The binding affinity of this variant for the 80-kDa IL-6 receptor was similar to that of wild-type IL-6. High affinity binding to CESS cells, however, was abolished, suggesting that the mutant protein is inactive because the complex of the 80-kDa IL-6 receptor and the mutant protein cannot associate with the signal transducer gp130. The human IL-6 antagonist protein may be potentially useful as a therapeutic agent.

Interleukin-6 (IL-6)[1] is a multifunctional cytokine playing a central role in host defense mechanisms (for reviews, see Refs. 1–3). IL-6 exerts its multiple activities through interaction with specific receptors on the surface of target cells (4, 5). The cDNAs for two receptor chains have been cloned and code for transmembrane glycoproteins of 80 and 130 kDa (gp130) (6, 7), which both belong to the large cytokine receptor superfamily (8–10). The 80-kDa IL-6 receptor (IL-6R) binds IL-6 with low affinity (K_d ~ 1 nM) without triggering a signal (11). The IL-6·80-kDa IL-6R complex subsequently associates with gp130, which then transduces the signal (7, 11). gp130 itself has no affinity for IL-6 in solution, but stabilizes the IL-6·80-kDa IL-6R complex on the membrane, resulting in high affinity binding of IL-6 (K_d ~ 10 pM) (7). Recently, it was found that gp130 is also a constituent of the receptors for leukemia inhibitory factor, oncostatin M, and ciliary neurotrophic factor (for recent review, see Ref. 14; Refs. 12 and 13).

In a variety of human inflammatory, autoimmune, and neoplastic diseases, abnormal IL-6 production is observed and has been suggested to play a role in the pathogenesis (reviewed in Refs. 3 and 15–17; Ref. 18). A causative role for IL-6 in the pathogenesis of multiple myeloma was indeed demonstrated by the observation that administration of an anti-IL-6 antibody to a patient with plasma cell leukemia could block myeloma cell proliferation in the bone marrow (19). Thus, inhibitors of IL-6 biological activity are useful to study its role in disease and could have broad therapeutic applications.

One strategy to neutralize IL-6 activity could be by inhibition of the ligand/receptor interaction with specific receptor antagonists. The feasibility of such an approach was recently demonstrated with a natural occurring receptor antagonist for interleukin-1 (for review, see Ref. 21; Ref. 20), which is currently being tested for utility in a variety of diseases (22). For IL-6, however, no natural receptor antagonist has been identified so far.

Previously, we have shown that neutralizing monoclonal antibodies (mAbs) to human IL-6 (hIL-6) recognize two distinct epitopes, designated sites I and II, on the hIL-6 molecule (23). We speculated that one of these sites might be involved in binding to the 80-kDa IL-6R, whereas the other might be involved in an interaction with gp130. According to the IL-6R model, IL-6 variants that bind normally to the 80-kDa IL-6R, but are somehow defective in inducing the interaction of the IL-6·80-kDa IL-6R complex with gp130, might be able to prevent heterodimerization of the receptor and function as receptor antagonists.

In this paper, we investigated the roles of sites I and II in IL-6/receptor interaction. We now show evidence for a role of site I in binding to the 80-kDa IL-6R and for site II in signal transduction because a biologically inactive hIL-6 mutant protein with mutations in site II could bind to the 80-kDa hIL-6R, but antagonized the biological activity of wild-type hIL-6. Binding experiments suggest that signal transduction cannot occur because the complex of the mutant protein and the 80-kDa IL-6R cannot associate with gp130. This is the first demonstration that it is possible to separate receptor binding from biological activity of hIL-6.

MATERIALS AND METHODS

Antibodies and Cytokines

The production and purification of the IL-6-specific mAbs have been described in detail (23). mAb B1 (LN1-73-10) was a kind gift of Dr. F. Di Padova (Sandoz Pharma, Preclinical Research, Basel, Switzerland). The purified wild-type rhIL-6 preparation used throughout these experiments as a standard is derived from *Escherichia coli* carrying the HGF7 plasmid (24). Purification of rhIL-6.HGF7 has been described (23). The specific activity of purified rhIL-6.HGF7 as determined in the mouse B9 assay is ~10^9 units/mg. Recombinant IFN-γ was a kind gift from Genentech (San Francisco).

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[1] The abbreviations used are: IL-6, interleukin-6 (prefix "rh" indicates recombinant human); IL-6R, interleukin-6 receptor; mAb, monoclonal antibody; IFN-γ, interferon-γ; CV, coefficient of variation; GH, growth hormone.

Expression Vectors and Bacterial Strains

Construction of the expression vector pUK-IL-6 has been described (25). For expression of rhIL-6 or rhIL-6 mutant proteins with this vector, *E. coli* DH5α (Life Technologies, Inc.) was used as the host. The bacteriophage T7 promoter vector pET8c and expression strain *E. coli* BL21(DE3) (26) were a kind gift of Dr. G. Pruyn (Department of Biochemistry, University of Nijmegen, Nijmegen, The Netherlands).

Expression Library Construction and Recombinant DNA and Sequencing Protocols

The vector pUK-IL-6 was used for construction of the library of rhIL-6 mutant proteins with randomly distributed substitutions of Gln-153–Thr-163. Construction of the library has been described in detail (27). Following transformation to *E. coli* DH5α, ~1000 colonies were obtained. DNA manipulation procedures were performed as described (23, 25). Nucleotide sequences of selected mutants (see below) were obtained with cDNA-derived oligonucleotide primers on double-stranded DNA by using the Sequenase kit (United States Biochemical Corp.).

Preparation of E. coli Extracts for Library Screening with mAbs

Four-hundred ampicillin-resistant colonies from the expression library were transferred to wells of 96-well flat-bottom microtiter plates (Nunc) containing 100 μl of LC amp medium (10 g of Bacto-Tryptone, 5 g of yeast extract, 8 g of NaCl, 2 ml of Tris base/liter supplemented with 100 μg/ml ampicillin (final concentration)). Following overnight culture at 37 °C, bacteria were lysed by addition of lysozyme to 1 mg/ml and further incubation for 30 min at 37 °C. One in 10 dilutions of the crude extracts in phosphate-buffered saline, 0.02% Tween 20, 0.2% gelatin was directly tested for reactivity in sandwich enzyme-linked immunosorbent assays with site I-specific mAb CLB.IL-6/8 (mAb8(I)) or site II-specific mAb CLB.IL-6/16 (mAb16(II)) coated on the plastic and biotinylated polyclonal goat anti-rhIL-6 as detecting antibody as described (23, 27). The nucleotide sequences were determined for mutant proteins that bound to mAb8(I), but not to mAb16(II).

Preparation and Quantification of E. coli Extracts for Biological Activity Measurements

To measure the biological activity of the mutant proteins that bound to mAb8(I), but not to mAb16(II), overnight cultures of *E. coli* DH5α carrying the mutant constructs were diluted 1:50 in 250 ml of LC amp medium and subsequently cultured to an absorbance at 550 nm of 1.5. Bacteria were harvested by centrifugation, resuspended in 5 ml of lysis buffer (phosphate-buffered saline, 1% Tween 20, 10 mM EDTA, 2 mM phenylmethylsulfonyl fluoride), and lysed by sonication. To solubilize rhIL-6-containing inclusion bodies, SDS was subsequently added to 1%. After 1 h of incubation at room temperature, SDS-insoluble material was removed by centrifugation (13,000 × *g* for 15 min). The biological activity of this SDS-solubilized material was directly measured in the mouse B9 and CESS assays starting from a 1:1000 dilution. At this dilution, the SDS did not affect the bioassays used. The IL-6 variant protein concentration of these preparations was determined by a competitive inhibition radioimmunoassay with IL-6-specific mAb CLB.IL-6/7 coupled to Sepharose 4B (Pharmacia LKB, Uppsala) and ^{125}I-rhIL-6 in the presence of 0.1% SDS. Unlabeled rhIL-6 served as a standard. mAb CLB.IL-6/7 binds heat- and SDS-denatured IL-6 and recognizes IL-6 residues Thr-143–Ala-146 as determined by pepscan analysis (28, 29).[2]

Expression and Purification of Mutant Proteins T163P and Q160E,T163P from E. coli

The IL-6 cDNA inserts from the vectors pUK-IL-6 T163P and pUK-IL-6 Q160E,T163P were subcloned in the vector pET8c, and the plasmids were transformed to *E. coli* BL21(DE3) for expression (26). The rhIL-6 variants were subsequently purified essentially as described (30). Briefly, the proteins were prepared from inclusion bodies by extraction with 6 M guanidine HCl, renaturation by dialysis against 25 mM Tris (pH 8.5), and purification by anion-exchange chromatography. The preparations were free of contaminating *E. coli*-derived proteins as judged by SDS-polyacrylamide gel electrophoresis followed by Coomassie Blue or silver staining. In some of the preparations, two bands were observed corresponding to the full-length mature protein and an ~10-amino acid shorter IL-6-derived degradation product. This degradation product does not significantly change the biological activity or the receptor binding of the full-length product because it generally constituted only 10–20% of the preparations. Moreover, carboxyl-terminal cleavage of only 5 amino acids of IL-6 reduces both the bioactivity and receptor binding affinity 1000-fold (23),[3] and amino-terminal cleavage of ~10 amino acids results in a product that behaves very similar to the wild-type molecule (25). Protein concentration was determined both by measuring the absorbance of the preparations and by the Bradford method (64) using bovine serum albumin as a standard. Bradford and $A_{280\ nm}$ data correlated best when assuming that the absorbance at 280 nm of a 10 mg/ml solution of IL-6 is 10.

IL-6 Bioassays

Mouse B9 Assay—The hybridoma growth factor activity of rhIL-6 and variants was measured in the mouse B9 assay as described (31).

CESS Assay—B cell stimulatory factor-2 activity of rhIL-6 variants was measured as described (32). IL-6-induced IgG1 production by the cells was subsequently measured in a sandwich enzyme-linked immunosorbent assay using a mouse mAb specific for human IgG1 (MH161-1M; from the Department of Immune Reagents, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service (C.L.B.), Amsterdam) in combination with a horse radish peroxidase-conjugated mouse mAb specific for human IgG (MH16-1ME; C.L.B.) with a human serum as a standard (H00-1234; C.L.B.). Enzyme-linked immunosorbent assay procedures were as described above.

HepG2 Assay—The hepatocyte stimulating activity of rhIL-6 variants was assessed by measuring the induction of C1 esterase inhibitor production by HepG2 cells as described (33). Following culturing to confluency (5×10^5 cells in 0.5-ml wells (Costar) in Iscove's modified Dulbecco's medium supplemented with 5% fetal calf serum, 5×10^{-5} M β-mercaptoethanol, 100 IU of penicillin, 100 μg/ml streptomycin, and 20 μg/ml human transferrin (Behringwerke, Marburg, Germany), HepG2 cells were washed twice and stimulated with serial dilutions of rhIL-6 or rhIL-6 mutant proteins for 48 h in the same medium in duplicate. In some experiments, cells were washed again after 24 h, and the cultures were continued for another 24 h in the presence of the same stimulus. This procedure results in a higher stimulation index. After the incubation period, C1 esterase inhibitor synthesis was subsequently measured by sandwich radioimmunoassay with anti-C1 esterase inhibitor mAb RII coupled to Sepharose 4B and ^{125}I-labeled sheep polyclonal anti-C1 esterase inhibitor IgG with normal human plasma as a standard as described (34).

Binding Experiments

The inhibitory effect of the mAbs on IL-6 binding to CESS cells was measured by using metabolically ^{35}S-labeled rhIL-6 as described (5, 35). The inhibitory effect of rhIL-6 and of the rhIL-6 Q160E,T163P mutant protein on ^{125}I-rhIL-6 binding to NIH-3T3 fibroblasts transfected with an 80-kDa IL-6R expression vector was measured as described (36). Inhibition of binding of ^{125}I-IL-6 to the soluble IL-6R was measured as follows. The extracellular ligand-binding domain of the 80-kDa IL-6R was expressed in NIH-3T3 fibroblasts as described (37). Culture supernatants of these cells were diluted 1:2 in 20 mM Tris-HCl (pH 7.5), 140 mM NaCl, 5 mM EDTA, 1% Triton X-100, 2 mM methionine, 0.01% NaN_3 and incubated with 5×10^4 dpm ^{125}I-IL-6 in the absence or presence of increasing concentrations of the rhIL-6 or Q160E,T163P protein for 2 h at 4 °C. ^{125}I-IL-6-soluble hIL-6R complexes were immunoprecipitated using an 80-kDa receptor-specific antiserum and protein A-Sepharose. Sepharose-bound radioactivity was subsequently measured with a γ-counter (37).

For Scatchard analysis of wild-type rhIL-6 and Q160E,T163P binding to CESS cells, rhIL-6 purified from Chinese hamster ovary cells expressing hIL-6 (a kind gift from Dr. D. Fischer, Interpharm Laboratories, Nes-Ziona, Israel) and Q160E,T163P, purified as described above, were labeled with ^{125}I-Bolton-Hunter reagent (4000 Ci/mmol, diiodinated; Amersham, Amersham, United Kingdom) essentially as described (4, 38). Briefly, 5 μg of rhIL-6 or mutant in 50 μl of borate buffer (50 mM $NaBO_3$, 0.02% Tween 20 (pH 8.5)) was added to 500 μCi of dried Bolton-Hunter reagent. rhIL-6 was incubated for 30 min at room temperature, and Q160E,T163P for 15 min at 0 °C with occasional mixing. Both reactions were stopped by addition of 100 μl of 5 mg/ml glycine in phosphate-buffered saline for 10 min. ^{125}I-Labeled rhIL-6 or

[2] J. P. J. Brakenhoff, M. Hart, F. Di Padova, and L. A. Aarden, manuscript in preparation.

[3] J. P. J. Brakenhoff, F. D. de Hon, V. Fontaine, E. ten Boekel, H. Schooltink, S. Rose-John, P. C. Heinrich, J. Content, and L. A. Aarden, unpublished results.

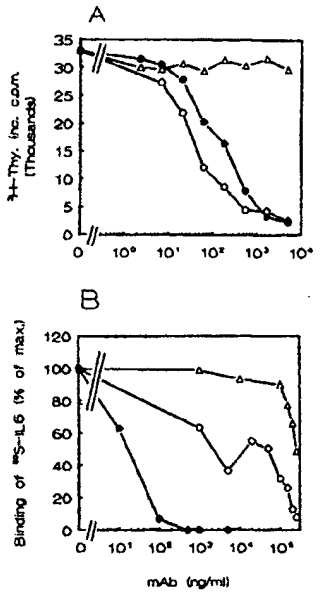

FIG. 1. **Differential effects of mAbB1(I) and mAb16(II) on IL-6 biological activity and receptor binding.** *A*, inhibition of mouse B9 hybridoma proliferation induced by 2 units/ml human monocyte-derived IL-6 with increasing concentrations of mAbB1(I) (●), mAb16(II) (○), and mAb14(III) (△). Data represent the mean of triplicate measurements. *B*, inhibition of ^{35}S-IL-6 binding to CESS cells. CESS cells were incubated with 80 pM ^{35}S-IL-6 in the absence or presence of increasing concentrations of the mAbs or unlabeled IL-6, and the amount of specifically bound IL-6 was measured as described under "Materials and Methods." Data represent the mean of duplicate measurements and are expressed as percent of maximum specific bound IL-6.

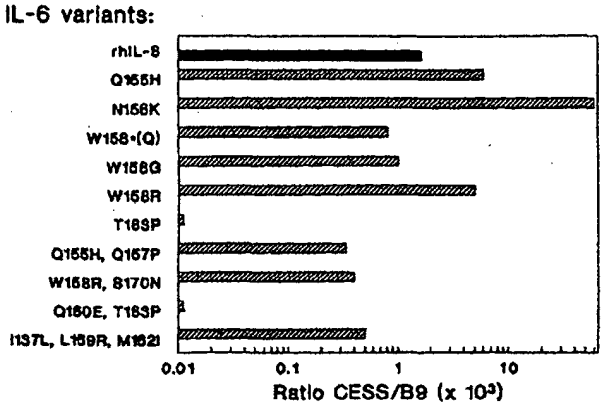

FIG. 2. **Biological activity of hIL-6 mutant proteins that do not bind to mAb16(II).** The hIL-6 site II mutants were selected with mAb8(I) and mAb16(II) from a hIL-6 expression library in *E. coli* as described in the text and under "Materials and Methods." The specific activity in mouse B9 and CESS assays was measured in SDS extracts of *E. coli* carrying plasmids encoding the rhIL-6 site II mutant proteins as described under "Materials and Methods." Data are expressed as the ratio of specific activity (units/microgram) in the CESS and mouse B9 assays. Values are derived from one of two experiments. *rhIL-6* indicates mature rhIL-6, and the *asterisk* in *W158*(Q)* indicates the TAG stop codon (due to the supE44 mutation in *E. coli* DH5α, a glutamine is incorporated in a fraction of the protein).

mutant was separated from ^{125}I-labeled products of low molecular mass by gel filtration chromatography on a PD-10 column (Pharmacia), sterile-filtered, and stored at 4 °C. The specific radioactivity of the preparations was determined by self-displacement analysis and was corrected for maximal binding capacity as described by Calvo *et al.* (39). The maximal binding capacity was 38% for ^{125}I-rhIL-6 and 16% for ^{125}I-Q160E,T163P. The specific activity of both preparations was ~160 Ci/mmol.

Binding assays were performed as described by Shanafelt *et al.* (40). Briefly, CESS cells were harvested, washed once, and resuspended in binding buffer (RPMI 1640 medium, 10% fetal calf serum, 50 mM Hepes, 0.02% NaN_3 (pH 7.4)). 2×10^6 viable cells/point were incubated with decreasing concentrations of ^{125}I-rhIL-6 or ^{125}I-Q160E,T163P in triplicate at 4 °C with continuous agitation for 3 h. Nonspecific binding was determined by including unlabeled rhIL-6 or Q160E,T163P as appropriate at a 500-fold excess. Cell-bound radioactivity was separated from free radioactivity by oil centrifugation, and bound and total radioactivities were counted with a Cobra 5010 γ-counter (40). The standard deviation of the triplicates was generally <5%. The equilibrium binding data were analyzed using the LIGAND program (41).

RESULTS

Site II mAb That Strongly Inhibits Biological Activity Has Little Effect on Receptor Binding of IL-6—The inhibitory effect of site I- and II-specific mAbs (23) was compared both in IL-6 bioassays and in IL-6 receptor binding experiments. Fig. 1*A* demonstrates the inhibitory effects of site I-specific mAb LN1-73-10 (mAbB1(I)) and site II-specific mAb CLB.IL-6/16 (mAb16(II)) on IL-6 biological activity in the mouse B9 hybridoma proliferation assay. The order of potency in this experiment was mAb16(II) > mAbB1(I), with EC_{50} values of ~40 and 180 ng/ml, respectively. A non-neutralizing control mAb (site III-specific mAb CLB.IL-6/14, mAb14(III) (23)) had no effect up to 5 μg/ml. This relative potency of the mAbs was observed in a number of experiments and was similar when tested on human cells.

To compare the inhibitory effects of the site I- and II-specific mAbs on biological activity with that on receptor binding of IL-6, human CESS cells (an Epstein-Barr virus-transformed B cell line) were incubated with ^{35}S-rhIL-6 in the presence of increasing concentrations of the mAbs. Fig. 1*B* shows that mAbB1(I) completely inhibited IL-6R binding at a concentration of 500 ng/ml, whereas mAb16(II) was unable to fully inhibit rhIL-6 binding even at a concentration of 250 μg/ml. At this concentration, the control mAb, mAb14(III), also inhibited receptor binding. So, whereas mAb16(II) inhibited biological activity of IL-6 more efficiently than mAbB1(I), also when tested with ^{35}S-rhIL-6 used in Fig. 1*B* (data not shown), it hardly interfered with the binding of IL-6 to CESS cells.

Construction and Analysis of Expression Library of Site II Mutant Proteins of IL-6—There are multiple ways in which mAb16(II) could inhibit biological activity without preventing receptor binding of IL-6. One of the possibilities was that site II, the epitope recognized by mAb16, is directly involved in signal transduction of IL-6 through interaction with gp130. Mutagenesis in this region could therefore result in the isolation of IL-6 mutant proteins defective in signal transduction, but not in receptor binding. In previous experiments, we had observed that binding of mAb16(II) to rhIL-6 was abolished by substitution of Trp-158 (27). However, this substitution protein was fully active when tested in the mouse B9 assay. Random mutagenesis of the region around Trp-158 was therefore performed to identify other residues involved in mAb16(II) binding, to perhaps uncover some that are also important to the biological activity of IL-6. A library of plasmids encoding rhIL-6 mutant proteins with randomly distributed substitutions of Gln-153–Thr-163 was constructed in the *E. coli* expression vector pUK-IL-6 (25). To select for variants with an intact receptor-binding site, the library was screened for mutant proteins that bound to a site I-specific mAb, but not to site II-specific mAb16. The nucleotide sequences of plasmids encoding mutant proteins with this phenotype were subsequently determined. The phenotype and the biological activity of the mutants are depicted in Fig. 2. Single substitutions of Gln-155, Asn-156, Trp-158, and Thr-163 disrupted the mAb16(II) epitope. From the double- and triple-substitution mutant proteins, it can be concluded that Leu-159, Gln-160, and Met-162 might also be im-

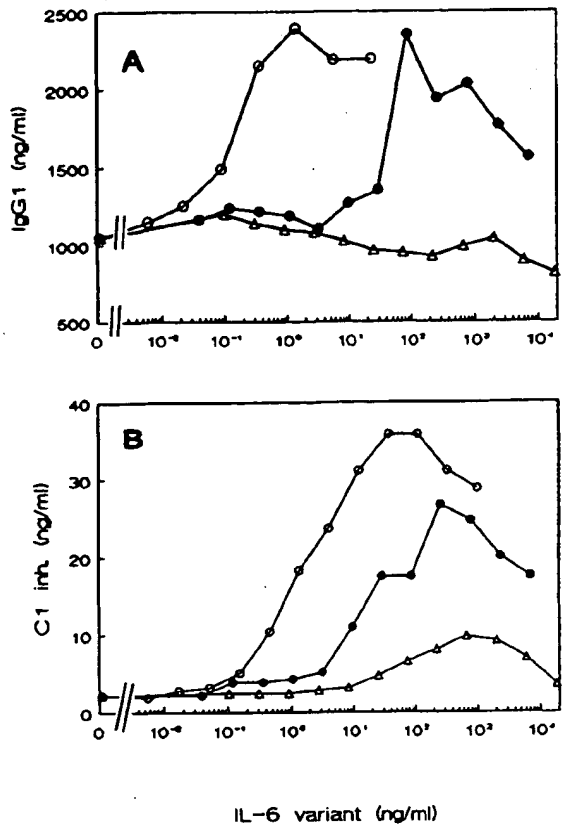

FIG. 3. **Activity of purified rhIL-6 variants in different bioassays for human IL-6.** Shown is induction by wild-type rhIL-6 (○), T163P (●), and Q160E,T163P (△) of IgG1 production by CESS cells (*A*) and C1 esterase inhibitor (*C1 inh.*) production by HepG2 cells (*B*). For each assay, one representative experiment is shown. Data points represent averages of three measurements. Assays were performed as described under "Materials and Methods."

portant for the mAb16 epitope, but this has yet to be confirmed by analyzing mutant proteins with single substitutions of these residues.

Some Site II Mutant Proteins Are Inactive on Human CESS Cells—The biological activity of crude extracts of the various mutant proteins was subsequently measured both in the mouse B9 assay and in IgG1 production by human CESS cells. All mutant proteins were biologically active in the mouse B9 assay. However, although very active in the mouse B9 assay, no activity could be detected in the rhIL-6.T163P and rhIL-6.Q160E,T163P mutant protein preparations on CESS cells (Fig. 2).

Selective Agonism of Q160E,T163P on Human Cells—To confirm the above observation, both IL-6 mutant proteins were purified and tested for biological activity on CESS cells and on a second IL-6-responsive cell line of human origin. Fig. 3 (*A* and *B*) shows representative dose-response curves of the mutant proteins in the two assays. In Table I, the specific activities of the mutant proteins in these assays are depicted, together with the specific activities in the mouse B9 assay. The T163P mutant protein was active in all assays, but with a lower specific activity than that of wild-type rhIL-6. As with the crude protein, the purified Q160E,T163P mutant protein again did not induce IgG1 synthesis by the CESS cells, not even when tested at a 10^5-fold higher concentration than the wild-type protein (Fig. 3*A*). At these high concentrations, this variant protein caused a weak increase in the production of the acute-phase protein C1 esterase inhibitor by HepG2 cells. This weak response was

TABLE I
Specific activity of purified rhIL-6 variants in IL-6 bioassays

rhIL-6 variant	Bioassay[a]		
	Mouse B9	CESS	HepG2
rhIl-6.HGF7	1.7	213	1.5×10^3
T163P	5	3.3×10^5	9.1×10^4
Q160E,T163P	11	$>10^7$	$>10^7$

[a] EC_{50} values (the concentration of IL-6 variant giving a half-maximal response in the assays), expressed in picograms/milliliter, were estimated graphically from dose-response curves of the purified proteins. Values represent averages of at least three experiments performed over a time span of several months. S.D. values varied from 14 to 90% of these values.

characterized by a strongly reduced maximal response as compared to wild-type rhIL-6 (Fig. 3*B*). On mouse B9 cells, the specific activity of Q160E,T163P was 6–7-fold lower than that of rhIL-6 (Table I).

Antagonism of IL-6 Action on CESS and HepG2 Cells by Q160E,T163P—We subsequently tested whether these mutant proteins were able to antagonize the biological activity of wild-type rhIL-6. Fig. 4 (*A* and *B*) shows that the Q160E,T163P mutant protein completely inhibited the wild-type IL-6 activity on CESS and HepG2 cells. In these experiments, 50% inhibition of IL-6 activity in CESS and HepG2 assays was observed with ~50 ng/ml and 1 μg/ml Q160E,T163P respectively, corresponding to 20- and 200-fold the concentration of wild-type rhIL-6 used to stimulate the cells. No antagonistic activity of the T163P mutant protein could be detected (data not shown). The inhibitory effect of Q160E,T163P on IL-6 activity in both the CESS and HepG2 assays could be reversed by high concentrations of wild-type rhIL-6, suggesting that the inhibitory mechanism is competitive inhibition of IL-6 receptor binding by Q160E,T163P (data not shown).

Specificity of Antagonism by Q160E,T163P—The production of the acute-phase protein C1 esterase inhibitor by HepG2 cells can be increased in response to both IL-6 and IFN-γ via separate mechanisms (33). To demonstrate the specificity of inhibition by the double-mutant protein, we tested whether Q160E,T163P could inhibit IFN-γ-induced C1 esterase inhibitor synthesis by the HepG2 cells. Because IFN-γ is more potent than IL-6 in this assay, we tested the effect of Q160E,T163P over a concentration range of IFN-γ. No inhibitory effects of Q160E,T163P were observed at any IFN-γ concentration tested. An example is shown in Fig. 5, demonstrating that the C1 esterase inhibitor synthesis induced by 5 ng/ml wild-type rhIL-6 was inhibited to background levels, whereas that induced by 1 ng/ml IFN-γ was unimpaired.

Binding to 80-kDa IL-6R by Q160E,T163P—The fact that the mutant protein Q160E,T163P could still be recognized by site I-specific mAb8 and that it could antagonize wild-type IL-6 activity on CESS and HepG2 cells suggested that the 80-kDa IL-6R-binding site of the mutant protein was still intact. To test this hypothesis, binding of this variant to the 80-kDa IL-6R (to both the membrane-bound and -soluble forms) was measured. In Fig. 6*A*, the capacity of Q160E,T163P to inhibit binding of ^{125}I-IL-6 to NIH-3T3 fibroblasts transfected with an expression vector encoding the 80-kDa IL-6R (36) was compared to that of wild-type rhIL-6. Fig. 6*B* shows the effect of the mutant protein and wild-type rhIL-6 on binding of ^{125}I-IL-6 to the soluble IL-6R. The results indicate that the mutant protein Q160E,T163P was 3–4-fold less efficient than wild-type rhIL-6 in inhibiting binding of ^{125}I-rhIL-6 to the 80-kDa IL-6R.

No High Affinity Binding of Q160E,T163P to CESS Cells—The above data suggest that Q160E,T163P is inactive on human cells because, although it can efficiently bind to the 80-kDa IL-6R, the complex of mutant and receptor is deficient in triggering signal transduction through gp130. To test

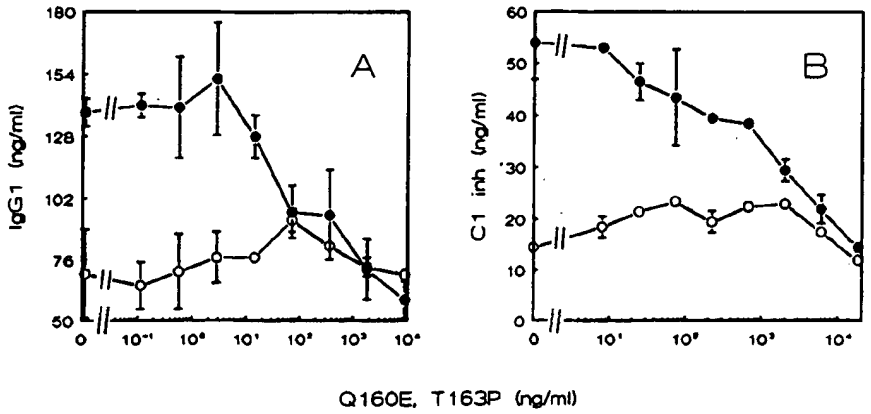

FIG. 4. **Antagonism of hIL-6 activity by Q160E,T163P.** *A*, CESS cells were incubated with increasing concentrations of Q160E,T163P in the absence (○) or presence (●) of 2 ng/ml wild-type rhIL-6. Data are expressed as means ± S.D. (n = 3). *B*, HepG2 cells were incubated for 48 h with increasing concentrations of Q160E,T163P with or without 5 ng/ml rhIL-6. Data points are means ± S.D. (n = 2). For both assays, one representative experiment of three is shown. *C1 inh.*, C1 esterase inhibitor.

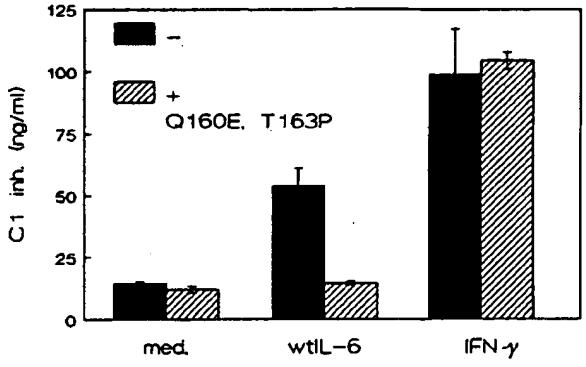

FIG. 5. **Q160E,T163P inhibits biological activity of hIL-6, but not of IFN-γ on HepG2 cells.** HepG2 cells were incubated with or without rhIL-6 (5 ng/ml) or recombinant human IFN-γ (1 ng/ml) in the absence or presence of 9 μg/ml Q160E,T163P. One of two experiments is shown. Data are means ± S.D. (n = 2). *C1 inh.*, C1 esterase inhibitor; *med.*, medium; *wt*, wild-type.

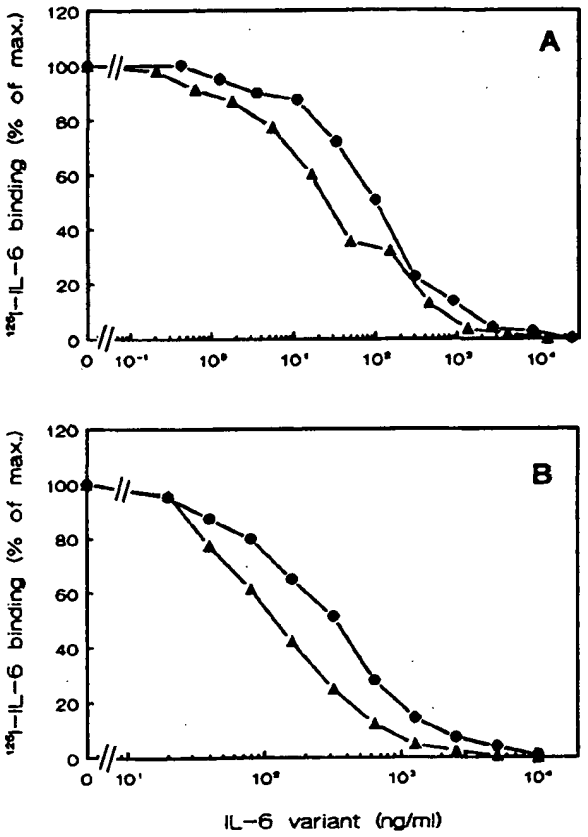

FIG. 6. **Q160E,T163P has affinity for 80-kDa IL-6R similar to that of wild-type rhIL-6.** *A*, competitive inhibition of 15 ng/ml (700 pM) ^{125}I-rhIL-6 binding to NIH-3T3 fibroblasts expressing the 80-kDa IL-6R with increasing concentrations of rhIL-6 (▲) or Q160E,T163P (●); *B*, inhibition of binding of 5 ng/ml (240 pM) ^{125}I-rhIL-6 to the soluble form of the 80-kDa hIL-6R with rhIL-6 and Q160E,T163P. Averages of duplicate measurements are shown for both assays. Assays were performed as described under "Materials and Methods."

whether Q160E,T163P could still bind with high affinity to human cells, we performed receptor binding experiments using ^{125}I-labeled Q160E,T163P and CESS cells. Coulie *et al.* (5) and Taga *et al.* (4) detected a single class of binding sites on CESS cells with K_d values of ~30 pM (1700 sites/cell) and 340 pM (2700 sites/cell), respectively. However, both with *E. coli*-derived ^{125}I-rhIL-6 (data not shown) and with Chinese hamster ovary cell-derived glycosylated rhIL-6, we always observed two binding sites. In Fig. 7, the results from representative experiments are displayed in Scatchard plots. Two binding sites were statistically significant for wild-type rhIL-6 (Fig. 7*A*; F = 26.14 (p = 0.001) for a one- *versus* two-site fit), with K_d values of 13 pM (coefficient of variation (CV) = 40%, 430 sites/cell (CV = 30%)) and 360 pM (CV = 31%, 3200 sites/cell (CV = 9%)). Q160E,T163P, however, exhibited only a single class of binding sites, with a K_d of 500 pM (Fig. 7*B*; CV = 59%, 7000 sites/cell (CV = 49%)). A two-site fit of the ^{125}I-Q160E,T163P binding data was not statistically significant (F = 0.03 (p = 0.9710) for a one- *versus* two-site fit). From this experiment, we concluded that Q160E,T163P is inactive on CESS cells because the binding of the mutant·80-kDa IL-6R complex to gp130 cannot occur.

DISCUSSION

In this paper, we show for the first time that receptor binding and signal transduction of hIL-6 can be uncoupled. This is evidenced by the following observations. 1) The Q160E,T163P mutant protein was inactive on CESS cells (the responses of Q160E,T163P are within the standard deviation of the background control), yet antagonized the biological activity of wild-type rhIL-6 on these cells. 2) The antagonist protein had an affinity similar to that of wild-type rhIL-6 for the 80-kDa IL-6R. According to the model for IL-6/receptor interaction, high

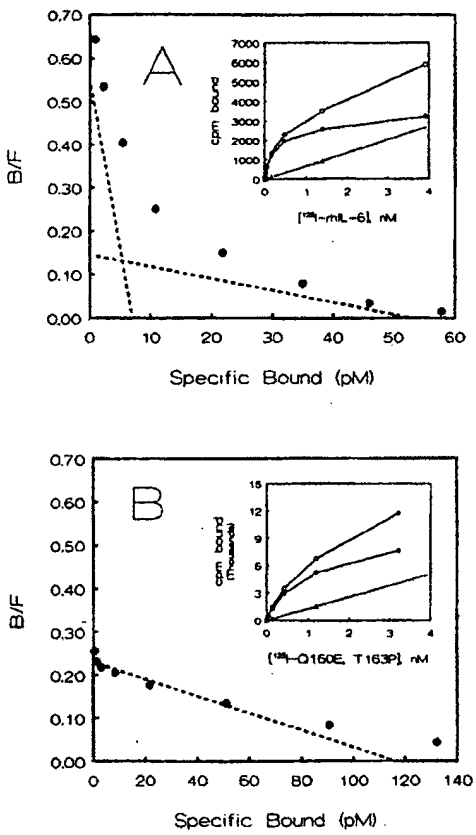

FIG. 7. **Scatchard analysis of rhIL-6 and Q160E,T163P binding to CESS cells.** Scatchard transformations of the binding of ^{125}I-rhIL-6 (*A*) and ^{125}I-Q160E,T163P (*B*) are shown. Data are derived from representative experiments with the same batch of CESS cells. Similar results were obtained in three independent experiments. *Dashed lines* represent the best fit values obtained from the LIGAND program (41) for these experiments. Wild-type rhIL-6 showed both high and low affinity binding (K_d = 13 pM (430 sites/cell) and 360 pM (3200 sites/cell); F = 26.14 (p = 0.001) for a two- *versus* one-site fit). Q160E,T163P exhibited a single class of binding sites, with a K_d of 500 pM (7000 sites/cell; a two-site fit was not statistically better than a one-site fit (F = 0.03 (p = 0.9710)). *Insets*, equilibrium binding isotherms (total bound (○), specific bound (●), and nonspecific bound (▲) ^{125}I-rhIL-6 or ^{125}I-Q160E,T163P) for the respective Scatchard plots are shown. *B/F*, bound/free.

affinity binding of the IL-6·80-kDa IL-6R complex to gp130 is a prerequisite for IL-6 signal transduction (7, 11). Our observations support this model because ^{125}I-Q160E,T163P bound only with low affinity to CESS cells, suggesting that the complex of the mutant protein and the 80-kDa IL-6R could not associate with gp130 to trigger a signal. Because IL-6 does not bind to gp130 in the absence of the 80-kDa IL-6R, it is not clear whether there is a direct interaction between IL-6 and gp130 in the complete receptor complex (11). Recent evidence from cross-linking experiments suggests that IL-6 and gp130 are in very close proximity in the IL-6·receptor complex (42, 43). Without structural information from crystallographic studies on the ligand-receptor complex, however, it is difficult to prove the existence of a contact and the possible role of IL-6 residues Gln-160 and/or Thr-163 therein.

Selective Agonism of Q160E,T163P—On HepG2 cells, at high concentrations of the Q160E,T163P mutant protein, we reproducibly observed a small partial agonist activity, characterized by a maximal response of ~10–20% of that of wild-type rhIL-6 (Fig. 3*B*). This might indicate that the antagonist·80-kDa IL-6R complex still exhibits some affinity for gp130, which we did not detect in the binding experiments with CESS cells. For the recently described hIL-4 (44) and mouse IL-2 (45) antagonist variants, the response also varied between cell lines studied and could be explained by differences in sensitivity of the cell lines to the respective wild-type cytokines: in the more sensitive cell lines, full receptor occupancy was not required to elicit a maximal response. Partial agonist activity of a mutant that was inactive on insensitive cells was explained by occupancy of spare functional receptors on the very responsive cell types, compensating for the receptor activation defects of the mutants (44, 45). This explanation cannot be applied to our results, however, because CESS cells, which were nonresponsive to the Q160E,T163P mutant, were more sensitive to wild-type rhIL-6 than HepG2 cells: for HepG2 cells, half-maximal stimulation was achieved at 70 pM rhIL-6, whereas 10 pM induced half-maximal stimulation of CESS cells (Table I). Although we have as yet no explanation for this observation, our results might be due to differences in IL-6 receptors on CESS and HepG2 cells. Pietzko *et al.* (43) recently showed that the gp130 molecule on HepG2 cells seems to differ in molecular mass from that on leukocytes and postulated the existence of a third receptor chain necessary for high affinity binding, in analogy with the IL-2 system (46). Furthermore, there might be differences in the relative numbers of high and low affinity receptors on CESS and HepG2 cells (Fig. 7*A*) (43).

In contrast to its activity on human cells, the Q160E,T163P mutant protein was nearly fully active on mouse cells, with a 6–7-fold reduced specific activity as compared to wild-type rhIL-6 in the mouse B9 assay. At first glance, this seemed to be due to the extremely high sensitivity of B9 cells to IL-6: half-maximal proliferation is induced by 0.08 pM, corresponding to a receptor occupancy of only 0.8%, assuming a high affinity K_d for the mouse IL-6R of ~10 pM. However, when we tested the Q160E,T163P mutant protein on the insensitive mouse plasmacytoma cell line T1165, which requires 10 pM IL-6 for half-maximal activation, as with B9 cells, the specific activity was ~15% of that of wild-type rhIL-6. This suggests that in the mouse system, the interaction of the Q160E,T163P·80-kDa IL-6R complex with gp130 is not as strongly affected as in the human system and that the double mutant should still be able to bind with high affinity to mouse cells. Further experiments are in progress to resolve the above issues.

Localization of Gln-160 and Thr-163 in Putative Tertiary Structure of hIL-6—Unfortunately, the tertiary structure of IL-6 is unknown. Based on homology comparisons, however, IL-6 belongs to the large group of cytokines that have an antiparallel four-α-helical bundle core structure similar to that of growth hormone, which include granulocyte/colony-stimulating factor, myelomonocytic growth factor, erythropoietin, and prolactin and also oncostatin M, leukemia inhibitory factor, and ciliary neurotrophic factor (8, 47, 48). For IL-6, the GH structure indeed seems to be a useful working model: Fig. 8 shows the localization of sites I and II in the hypothetical three-dimensional model for hIL-6 based on the GH structure. mAbB1(I) was shown to recognize residues at both the amino and carboxyl termini of IL-6, which fits with close proximity of helices A and D in the model (23). Site I and II-specific mAbs are capable of forming a sandwich with monomer rhIL-6 in enzyme-linked immunosorbent assays, which also agrees with the model (23). Site I on IL-6 and residues close to it are likely to be the 80-kDa receptor-binding site. 1) Site I-specific mAbs strongly inhibit receptor binding, and 2) site I colocalizes with Ser-178–Arg-185, which were recently shown to be essential for biological activity and receptor binding of IL-6 (23, 49–53). Site I also maps closely to a region essential to bioactivity in the NH_2-terminal α-helix (Ile-30–Asp-35) (25, 29). This region on hIL-6 partially colocalizes with binding site 1 on human GH,

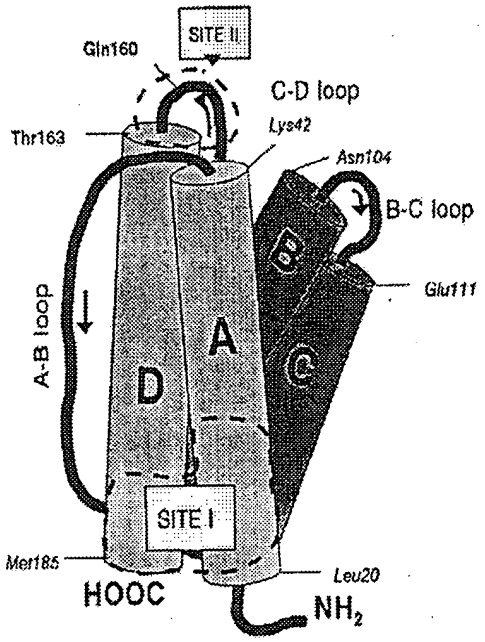

FIG. 8. **Locations of sites I and II, Gln-160, and Thr-163 in putative tertiary structure of hIL-6.** The structure is based on that of GH (62, 63) and is adapted from Ref. 8. Only the helix bundle core and loop connectivity are shown. IL-6 residues predicted to be at the end of the α-helices are derived from Ref. 47. *Helix A*, Leu-20–Lys-42; *helix B*, Glu-82–Asn-104; *helix C*, Glu-111–Lys-132; *helix D*, Thr-163–Met-185. Localization of site I is derived from Ref. 23, and that of site II from Fig. 2.

which was identified as a patch consisting of three discontinuous segments: the loop between GH residues 54 and 74 (the A-B loop), the COOH-terminal half of helix D, and, to a lesser extent, the NH_2-terminal region of helix A (54, 55). Whether the A-B loop in hIL-6 is also part of the 80-kDa binding site is as yet unknown.

Gln-160 and Thr-163 are located in the C-D loop and at the beginning of helix D, respectively, and are part of site II. This region does not colocalize with site 2 on human GH, which consists of residues near the NH_2 terminus, and on the hydrophilic faces of helices A and C (56). It does colocalize, however, with one of the regions of greatest similarity between neuropoietic and hematopoietic cytokines (including IL-6, leukemia inhibitory factor, oncostatin M, and ciliary neurotrophic factor), which was called the "D1 motif" (47). Because gp130 is part of the receptor complex of IL-6 (12, 13) and is essential for signal transduction by IL-6, ciliary neurotrophic factor, leukemia inhibitory factor, and oncostatin M (57, 58), it would be interesting to test the effect of mutations in the corresponding D1 regions of these cytokines on biological activity and gp130 interaction.

Conclusion—In this report, we described for the first time the isolation of an IL-6 analog that could antagonize the activity of wild-type hIL-6 in some *in vitro* bioassays. The dose range in which this molecule inhibited IL-6 activities in these bioassays is similar to that reported for the natural occurring IL-1ra (59). The pleiotropy of IL-6 and the broad distribution of its receptors make selective therapeutical application of receptor antagonists difficult to envision. However, the observation that it is possible to isolate an IL-6 variant that is active in some (but not all) bioassays may point to the possibility of isolating selective agonists of IL-6 that retain useful activities, but antagonize IL-6 activity where desired. Otherwise, combination of an IL-6 antagonist with other cytokines that share some (but not all) of the functions of IL-6 might in part restore the useful activities of IL-6. Detailed information concerning the *in vivo* sites of production and the activities of IL-6 and cytokines with similar activities is required before considering these options. Development of IL-6 inhibitor strategies seems very appropriate regarding the speed with which diseases are discovered in which IL-6 seems to play a role (60, 61).

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Epitope peptides from interleukin-6 receptor which inhibit the growth of human myeloma cells

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ABSTRACT. A panel of monoclonal antibodies against the soluble IL-6 receptor was used to search for linear epitopes by a Pepscan analysis. Two such epitopes were found and the corresponding peptides were synthesized chemically. The peptides were active to inhibit the IL-6 dependent growth of human multiple myeloma cell line and the effect of IL-6 on growth of murine hybridoma cells. The epitope-defined, antagonist peptides reduced the transduction of the IL-6 signal which activates binding of Stat transcription factors to specific enhancers, but did not affect IL-6 binding. These effects were not seen with several other peptides from the IL-6 receptor sequence. A computer three-dimensional model of the IL-6 receptor complex was built and indicates that the antagonist peptides define one of the two possible sites of interaction between the domain-II of the IL-6 receptor molecule and that of the gp130 molecule within the hexameric receptor assembly.

Key words: Multiple myeloma, Cytokine Receptors, growth inhibitors, Interleukin-6 receptor, gp130 transducer.

INTRODUCTION

The IL-6 receptor system comprises two membrane proteins: gp80 or IL-6 receptor (IL-6R) which binds IL-6 at low affinity, and gp130 required for high affinity binding and signal transduction [1, 2]. A 55 kDa soluble form of IL-6R (sIL-6R), lacking the transmembrane domain, is present in serum and excreted to urine [3, 4]. Human cells also secrete sIL-6R produced through differential mRNA splicing [5, 6]. A particular feature of the IL-6 receptor system is that the various forms sIL-6R act as agonists of IL-6 in human and in murine cells [4-8]. Since sIL-6Rs lack the intracytoplasmatic (IC) domain, their agonist activity suggests that they interact with the extracellular (EC) portion of gp130. In contrast, the soluble gp130 inhibits IL-6 action indicating that IL-6 signal transduction does depend on the IC domain of gp130 [9]. Binding of the IL-6/IL-6R complex induces the dimerization of gp130 and activation of intracytoplasmatic tyrosine kinase [10]. Jak/Tyk kinases and Stat transcription factors are associated with the IC domain of gp130 [11, 12]. Stat1 or Stat3, as homodimers or in combinations with other proteins participate in IL-6 signaling [13-15]. Four such Stat complexes form on palindromic promoter sequences of IL-6 inducible genes such as IRF-1 when IL-6 is added to a variety of cell types [13,14], and some of these complexes are also involved in the activation of certain acute phase response genes in liver cells [15].

Because of its agonist properties, it would be important to understand which regions of sIL-6R are engaged in interactions with IL-6 and gp130. Structurally, IL-6R as well as gp130, contain an EC domain which belongs to the class 1 cytokine receptor family [16, 17]. DeVos *et al.* [18] reported the crystallographic structure of a member of this family, the human growth hormone receptor (GHR), showing that the cytokine receptor has two domains (N-proximal or domain-I, and C-proximal or domain-II), each with seven β-strands forming two antiparallel β-sheets, one three-stranded (β-strands A, B, E) and one four stranded (β-strands C, C', F, G), as predicted by Bazan [16]. In this structure, two GHR molecules interact each with different sites on the growth hormone ligand and also interact with each other forming a trimer in which the two GHR homodimerize. However, the IL-6 receptor complex must differ from the structure proposed for the growth hormone receptor, since two gp130 molecules have to dimerize for signaling [10]. Experimental evidence has been provided for a hexameric structure containing two molecules of IL-6, two of gp130 and two of IL-6R [19]. In support of this model, it was recently shown by mutations in IL-6 that this ligand interacts with one IL-6R and with two gp130 molecules [20]. Therefore, IL-6R is expected also to interact with two gp130 molecules in addition to IL-6 binding. Previously, mutation analysis of the cytokine receptor domain of IL-6R has demonstrated two types of functional residues, some being essential for IL-6 binding and

others, mainly clustered at residues 280-288, being essential for interaction with gp130 and signal transduction [21]. As another approach to identify sites of interactions within IL-6R, we have identified linear sequences corresponding to the epitopes of two monoclonal antibodies (McAB) against IL-6R which interfere with the receptor action. The epitope peptides identified by a Pepscan analysis [22] define a distinct site of interaction in the domain II of the IL-6R and block IL-6 action on human myeloma cell growth. Our results are in partial agreement with the results of Grube and Cochrane [23] who analyzed a series of peptides chosen randomly along the IL-6R structure.

MATERIAL AND METHODS

Pepscan analysis. Decapeptides were synthesized on a 96-spot membrane using the SPOTS kit (Cambridge Research Biochemicals), purchased from Euromedex (France). The first peptide was residues 123-132 of IL-6R and each consecutive peptide was offset by two residues from the preceding one. The membrane was reacted successively with anti-IL-6R McABs from the pannel described in detail previously [24], used as immunoglobulins at concentrations of 10 - 20 µg/ml. As second antibody, binding of sheep anti-mouse IgG, horseradish peroxidase conjugated (1:5000 dilution) was measured by enhanced chemoluminescence (ECL kit, Amersham Radiochemicals, UK). Candidate peptides were synthesized in an Applied Biosystem peptide synthesizer. The list of peptides is given in Table 1.

Assay of human myeloma XG-1 cell growth. XG-1 cells [25,26; kind gift of Dr Lucien Aarden] were cultured in Iscove's modified Dulbecco's medium (IMDM, Gibco-BRL) with 5% fetal calf serum (FCS), 50 µM β-mercaptoethanol, 20 µg/ml transferrin, penicillin, streptomycin and 1 ng/ml of pure recombinant human IL-6 from CHO cells (InterPharm Labs, Israel, Ares-Serono Group), prepared and assayed as described [14]. For IL-6 dependent growth assay, semi-confluent cultures at passage 15-20 were depleted of IL-6 by two washes in medium followed by 4 hours culture at 37°C in medium with 10% FCS. Cells were then plated in 12-wells Costar plates at 5 x 10^4 cells/ml in fresh medium with serial 1:5 dilutions of IL-6. After 3 days at 37°C, triplicate aliquots of 0.1 ml of each well were transferred to a 96-well microplate (Nunc), pulsed 3-hours with ^{3}H-Thymidine (1 µCi per well, 25 Ci/mmol, Amersham), collected on 3 MM paper disks and counted by scintillation. Half maximum growth (^{3}HThymidine incorporation) was typically at 20 pg/ml IL-6 in 5% FCS and 40 pg/ml IL-6 in 10% FCS. The synthetic soluble peptides were tested under these conditions and were added at 100 - 250 µg/ml, to the Costar plate wells 15 minutes prior to IL-6.

Assay of murine hybridoma B9 cell growth. Murine hybridoma B9 cells [27] were cultured in RPMI 1640 (Biolabs, Israel) with 10% FCS, 50 µM β-mercaptoethanol in the presence of 1 - 2 ng/ml IL-6. Cells were depleted of IL-6 as above and then distributed into microtiter plate wells (10^4 cells per 0.2 ml) with 0, 30, 125 and 500 pg/ml IL-6. After 3 days culture at 37°C, 20 µl of alamar Blue (a colorimetric growth indicator based on oxido-reduction, BioSource, Camarillo, CA) was added to each well and incubation continued for 3 hours or until the color changes are stable. Color was measured in a microplate ELISA reader at 530/630 nm. A calibration curve correlating living cell number (trypan blue dye exclusion) and OD530 nm was used to verify linearity. Each assay was run in quadruplicates, the peptides being added at 62.5-250 µg/ml about 15 minutes before IL-6 addition. The mean IL-6 dependent difference in OD530 was taken as 100% growth. Data are given for 500 pg/ml IL-6. Statistical analyses were by two-tailed Student's t-test with an Instat computer program.

Assay of signal transduction. B9 cells at 2.5 x 10^5 cells/ml were starved of IL-6 by two washes in RPMI and 4 hours culture as above without IL-6. Each reaction was done in 25 ml (10^6 cells/ml) with or without peptides (250 µg/ml) and 500 pg/ml IL-6 for one hour at 37° C. After washing with phosphate buffered saline (PBS), cell pellets were frozen in liquid nitrogen, then lyzed for extraction of nuclear factors as described [13]. The nuclear extracts (2 µl) were incubated with ^{32}P-end-labeled the oligodeoxy-ribonucleotide probe pIRE/IRF-1, representing the IL-6 responsive enhancer of the IRF-1 gene [13, 14]. The DNA electrophoretic mobility shift assays were carried out as detailed [13] and the amount of IL-6 induced pIRF-A complex quantified in a Phospho-Imager (FujiX BAS 1000) as before [14]. An internal control was used to insure equivalent protein concentrations.

Computer model. The complex formed by the human IL-6, IL-6R and gp130 molecules was built by analogy to the complex of human growth hormone (GH) - growth hormone receptor (GHR) whose X-ray structure has been solved [18] and deposited as entry 3HHR in the Protein Data Bank [28]. The model of IL-6 was built by homology to bovine G-CSF (entry 1BGD in the Protein Data Bank), as previously aligned [29]. The sequence alignements of Bazan [16] were used for the cytokine receptor domains. The model of IL-6R includes aminoacids 117 - 314 of the complete sequence [1], and that of gp130, aminoacids 125 - 330 of the complete sequence [2]. The models of each of the three structures were built separately using the program Homology (Biosym Technologies, San Diego, CA) and the energy minimized using the program Encad [30]. The positions of the Cα atoms were strongly restrained in the minimization to preserve the overall fold of the polypeptide chain. The three models were assembled into a complex by analogy to the GH-GHR trimeric complex, and the structure was then minimized with restrain on the Cα positions. A hexameric complex was constructed by manual docking of two trimeric complexes against one another.

RESULTS

Definition of linear peptide epitopes of McAB 34.4 and 50.6. A pannel of monoclonal antibodies against sIL-6R, which variably inhibit the binding of IL-6 to sIL-6R, was generated [24]. Several of these McAB, among which 34.4 and 50.6, were shown to inhibit the binding of IL-6 to human cells as well as IL-6 biological activity [4]. We examined eight McAB of this pannel for the recognition of linear peptides from the IL-6R extracellular domain by using the Pepscan approach [22]. A series of 96 decapeptides were synthesized which span residues 123 - 322 of the IL-6R sequence [1], this region being predicted to be the cytokine receptor domain of IL-6R [16]. Each consecutive peptide was offset by 2 aminoacids from the previous one, yielding a nested, overlapping library. The membrane carrying the 96 peptides was reacted with McAB 34.4 (18 µg/ml) and stained with horseradish peroxidase-conjugated secondary antibody followed by enhanced chemiluminescence (ECL). This semi-quantitative detection method allowed to compare the intensity of the reaction between the antibody and the diverse peptides. As indicated in Fig. 1, reaction was observed with only 4 consecutive peptide spots (67, 68, 69 and 70) corresponding to aminoacids 255 - 264, 257 - 266, 259 - 268 and 261 - 270 of IL-6R. From these results, it appears that the core of the epitope for McAB 34.4 is RSKT (261 - 264).

When the 96-peptide library of IL-6R was tested with McAB 50.6, spots 64 and 65 reacted most strongly indicating sequence 251 - 258 as the core epitope for this second neutralizing antibody (Fig. 1). The other McABs tested (20.2, 32.3, 17.6, 38.8, 48.8) in our pannel [24] did not give clear signals on this library of linear peptides, and probably correspond to non-linear epitopes.

Effect of epitope peptides on growth of human myeloma XG-1 cells. The aminoacid sequences forming the linear epitopes recognized by neutralizing McAB 34.4 and 50.6 may be involved in some protein-protein interaction required for IL-6 action. Hence, short peptides with such sequences could mimick these interactions and interfere with the function of the IL-6 receptor system. We, therefore, synthesized two peptides: L^{255} - V^{270} (peptide 1063, corresponding to McAB 34.4 epitope) and S^{247}-E^{260} (peptide 1062, around the McAB 50.6 epitope) as indicated in Fig. 1. Other peptides selected in potentially important regions of sIL-6R, and a control peptide, were similarly

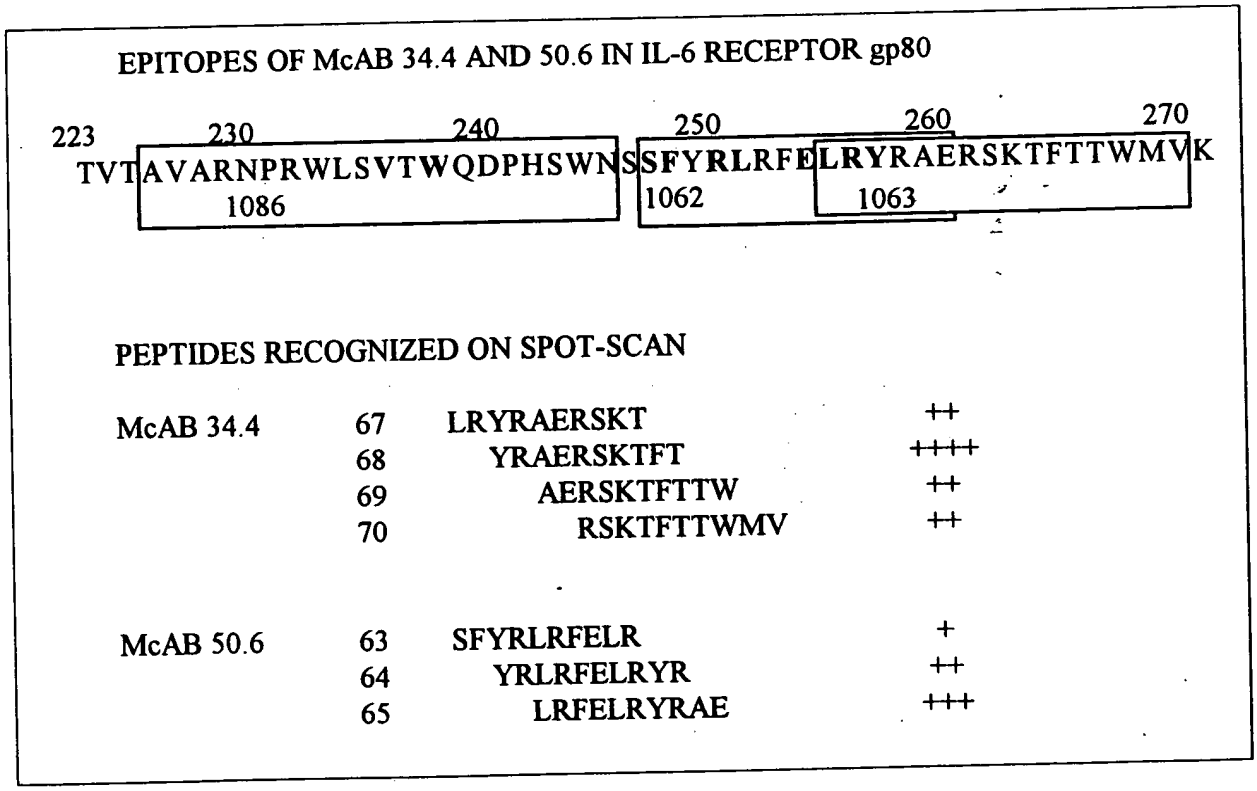

Figure 1
Epitopes of McAB 34.4 and 50.6 in IL-6 receptor gp80
Reaction of McABs 34.4 and 50.6 with decapeptides on the Spot-membrane. Intensity of signal is shown by + signs. The relevant segment of human IL-6R is shown and the limits of three synthetic peptides tested as antagonists is shown by boxes. The bold letters indicate aminoacids whose mutation affects function [21].

Table 1
Peptides tested for inhibition of IL-6 activity and their coordinates in IL-6R

IL-6R Peptide Coordinates	Peptide Number	Sequence	Remarks
S^{247} - E^{260}	1062	SFYRLRFELRYRAE	McAV 50.6 epitope
L^{255} - V^{270}	1063	LRYRAERSKTFTTWMV	McAB 34.4 epitope
C^{277} - G^{299}	983	CVIHDAWSGLRHVVQLRAQEEFG	domain-II strands E,F
V^{290} - Q^{300}	1064	VQLRAQEEFGQ	IL-6 binding
S^{125} - G^{135}	1067	SPLSNVVCEWG	domain-I strand A
A^{226} - N^{245}	1086	AVARNPRWLSVTWQDPHSWN	domain-II A-B loop
	903	PGHRYRDQQTQTSFSEEPOQSSQLLPC	control peptide

prepared (Table 1). The peptides were then tested for their ability to interfere with IL-6 action on the growth of a human myeloma cell line XG-1 whose growth is dependent on IL-6, as demonstrated by addition of anti-IL-6 antibodies and of exogenous IL-6 [25, 26].

The dose-dependence of CHO rIL-6 on growth of XG-1 cells was calibrated by measuring ^{3}H-Thymidine incorporation after 3 days of culture (see Methods). Table 2 shows the stimulation of ^{3}H-Thymidine incorporation by IL-6 (40 pg/ml) in XG-1 cells at 10% FCS. Addition of peptides S^{247}-E^{260} and L^{255} - V^{270} (100 µg/ml) reduced the IL-6 induced proliferation of the cells. The overall growth in presence of IL-6 was reduced by 42 - 65%. Another IL-6R peptide (V^{290} -Q^{300}) had only a marginal effect. An experiment carried out under more stringent growth conditions (20 pg/ml IL-6 and 5% FCS) and with higher peptide concentrations (250 µg/ml) is shown in **Fig. 2.** A strong inhibition of over 90% in the overall growth in presence of IL-6 was obtained with peptide L^{250} - V^{270} (1063). There was also partial growth reduction by S^{247} - E^{260} (1062) and another peptide A^{226} - N^{245} (1086, see Table 1 for sequence), whereas a series of other peptides from IL-6R were not more active than an unrelated control peptide 903 (Fig. 2). Therefore, under various experimental conditions, the peptides defined by the McAB epitopes interfere with the growth of an IL-6 dependent cell line derived from a human Multiple Myeloma patient. No such effect was seen in the U266 cell line whose growth is not IL-6 dependent (not shown).

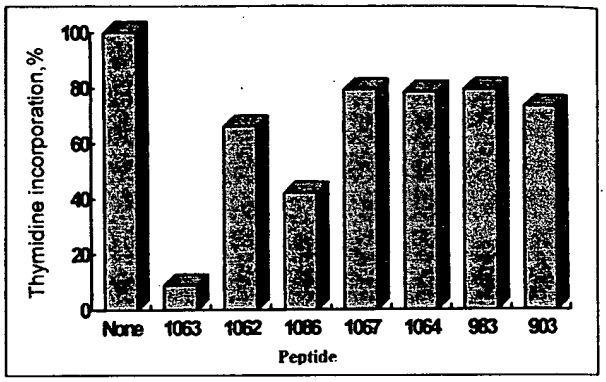

Figure 2
Effect of IL-6R epitope and other peptides on human myeloma XG- 1 cell growth.
Thymidine incorporation was measured 3 days after seeding with IL-6 (20 pg/ml) without or with indicated peptides at 250 µg/ml. For peptide coordinates and sequences, see Table 1.

Inhibition of IL-6 dependent murine hybridoma B9 cell growth. The plasmacytoma derived B9 murine hybridoma has a specific and very sensitive dependence of IL-6 for growth [27]. We developed a convenient assay for B9 cell growth which measures cell density by a redox indicator dye, alamar Blue, allowing spectroscopic analysis in microplates. Over a wide range of IL-6 concentrations (0 - 1,000 pg/ml) there was close parallelism between the number of

Table 2
Inhibition of human myeloma cell growth by epitope peptides

	Human Myeloma XG-1 Cell Growth with Peptide			
	none	S^{247} - E^{260}	L^{255} - V^{270}	V^{290} - Q^{300}
	^{3}H-Thymidine incorporation, cpm x10^{-3}			
No IL-6	6.4 ± 1.6	8.3 ± 0.3	8.7 ± 2.5	10.1 ± 0.3
IL-6, 40 pg/ml	43.6 ± 0.9	15.1 ± 3.3	25.2 ± 0.2	39.1 ± 1.5
IL-6 dpdt (%)	37.2 (100)	6.8 (18.2)	16.5 (44.3)	29.0 (78)

Peptides used at 100 µg/ml. Average of triplicate experiments.

Table 3
Inhibition of IL-6 dependent hybridoma B9 cell growth by epitope peptides

PEPTIDE	IL-6 DEPENDENT HYBRIDOMA B9 CELL GROWTH		
	62.5 µg/ml	125 µg/ml	250 µg/ml
	Per cent of IL-6 dependent cell growth ± SD		
L^{255} - V^{270} (1063)	71 ± 9 (p = 0.001)	45 ± 16 (p < 0.001)	2 ± 1 (p < 0.0001)
S^{247} - E^{260} (1062)	100± 5 (n.s.)	15 ± 9 (p < 0.0001)	
control (903)	97 ± 4 (n.s)	95 ± 9 (n.s)	89 ± 15 (n.s.)

Statistical analyis of 5 experiments using two-tailed Student's t-test in comparison to no peptide. The comparison of B9 cell growth with peptide L255 - V270 at 125 µg/ml to control peptide 903 at 250 µg/ml was also significant at p = 0.004.

cells counted per ml and the optical density at 530 nm resulting from the alamar Blue dye. An OD530 of 0.2 corresponded to about 5 x 10^5 B9 cells/ml. In a typical experiment the OD530 was at 3 days of 0.02 without IL-6 and 0.25 with 500 pg/ml CHO rIL-6.

To assay the peptides, the hybridoma B9 cells were first starved for 5 hours of IL-6, and then seeded in a microplate with 500 pg/ml IL-6 and with different concentrations of peptides. After 3 days the cell density was measured by staining with alamar Blue dye and the IL-6 dependent growth was calculated. As seen in Fig. 3, peptide L^{255} - V^{270} (1063) produced a dose-dependent inhibition of the B9 cell growth, reducing the growth to levels similar to those observed without IL-6. Growth inhibition was also observed with peptide S^{247} - E^{260} (1062) but with a different dose-curve. A control peptide (903) had no such effect. The activity of these peptides was tested in 5 separate experiments. The variations were small and the inhibition by peptide L^{255} - V^{270} was statistically significant at the 3 doses tested (Table 3). Inhibition by peptide S^{247} - E^{260} was also significant whereas control peptide 903 produced no significant changes.

Among the other peptides tested, peptide A^{226} - N^{245} (1086) also inhibited the effect of IL-6 on B9 cell growth, whereas S^{125} - G^{135} (1067) had almost no effect (Fig. 3). In addition, peptides V^{290} - Q^{300} and C^{277}-G^{299} had no significant effect (not shown).

Effect of epitope peptides on signal transduction by the IL-6 receptor complex. An early effect of IL-6 on B9 cells is the activation of transcription factors Stat1 and

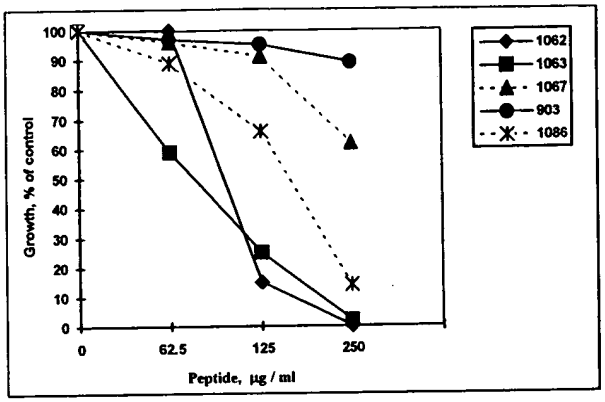

Figure 3
Effect of IL-6R epitope and other peptides on the IL-6-dependent growth of murine hybridoma B9 cells.
Cell growth was measured by alamar blue dye assay at 3 days after seeding with 500 pg/ml IL-6 and indicated peptides concentrations. For peptide coordinates and sequences, see Table 1.

Stat3 for binding to palindromic enhancer sequences pIRE, such as found in the promoter of the Interferon-Regulatory Factor 1 (IRF-1) gene [13, 14] . Extracts of cells treated 1 hour by IL-6 form a major complex called pIRF-A with the labeled pIRE oligonucleotide, which was quantitated by DNA-mobility shift gel electrophoresis. The induction of the pIRF-A complex by IL-6 was markedly inhibited by the addition of peptides L^{255} - V^{270} and S^{247} - E^{260} to IL-6 during the 1 hour cell stimulation period, in comparison to the effect of a non specific peptide (Table 4). This

Table 4
Effect of epitope peptides on IL-6 signaling

Peptide added	pIRF-A DNA-protein complex	
	Fold increase by IL-6	Inhibition by peptide
None	8.4	-
L^{255} - V^{270} (1063)	3.0	64.3%
S^{247} - E^{260} (1062)	2.9	65.5%
control (903)	6.0	28.6%

Peptides (250 µg/ml) were added with IL-6 (0.5 ng/ml) for 1 hour.

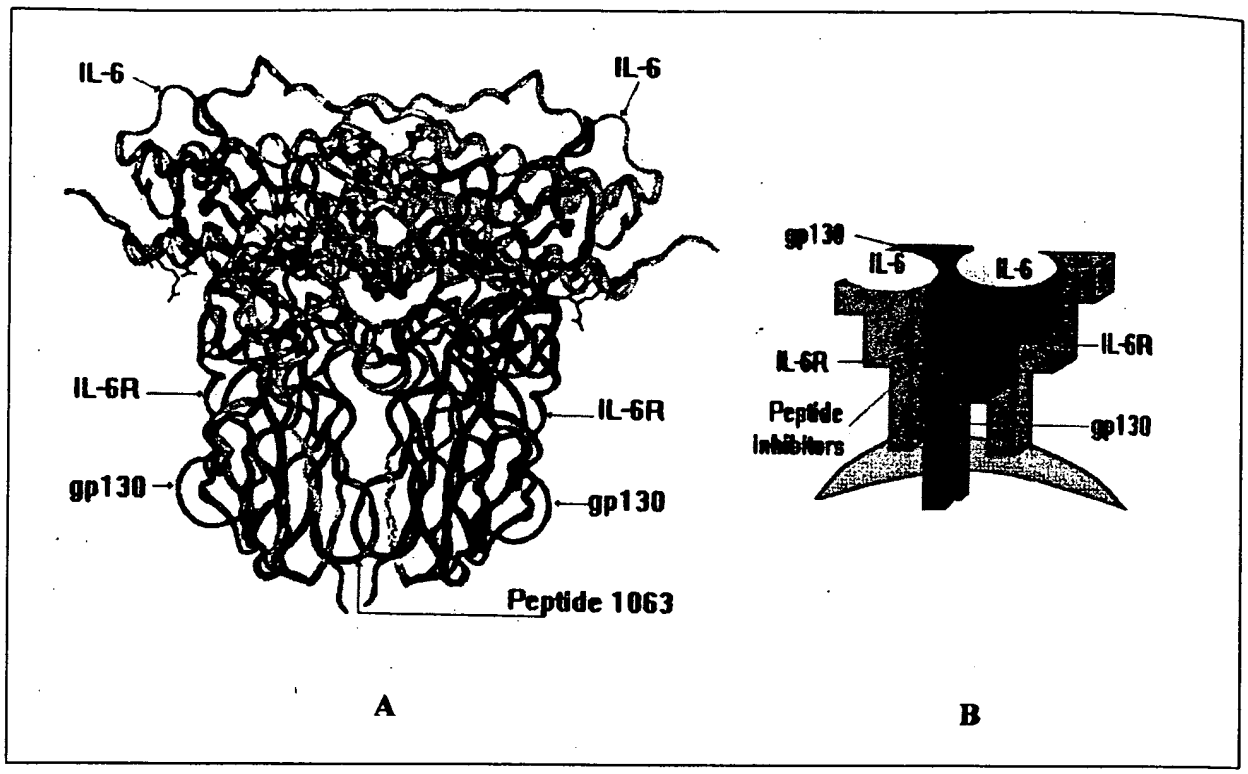

Figure 4
Computer 3-D model (A) and scheme (B) of the hexameric IL-6 receptor complex showing the cytokine receptor family domains of IL-6R and gp 130.

(A) In the 3-D model, two IL-6 molecules are seen on top (yellow and grey). On the left side, IL-6R (red) is in front of a gp130 (blue). In this IL-6R chain the core epitope peptide of McAB 34.4 is shown in green-red-green (peptide L^{255}-V^{270}, 1063). The overlapping peptide from McB 50.6 epitope (S^{247}-E^{260} or 1062) is shown in grey, extending in the following green area. The loop carrying the epitopes is the β-strand C-C' junction of the 4-stranded β-sheet of domain-II. On the right side, gp 130 (dark blue) is in front of IL-6R (dark red). A site on gp130 which contacts the region of peptide 1063 is shown in yellow and is on strand A of the domain-II of gp130. For details see text. (B) In this scheme, IL-6R is in red and gp130 in blue. The black arrows show interactions 1 and 2 of IL-6R and gpl 30 as given in Table 5. The antagonist peptides correspond to interaction 2.

indicates that the peptides can affect the receptor signaling leading to activation of the Stat transcription factors. In contrast, we found no effect of peptides L^{255} - V^{270} or S^{247} - E^{260} on binding of radio-iodinated IL-6 to recombinant sIL-6R by cross-linking assay [6]; only peptide S^{125} - G^{135} gave a 30% inhibition in this assay (not shown).

DISCUSSION

The pepscan analysis of the extracellular domain of the IL-6 receptor gp80 molecule revealed two linear epitopes recognized by McABs 34.4 and 50.6 which are known to neutralize IL-6 binding and action on cells. The antigenic determinants of these two McABs were overlapping, whereas other McABs of our pannel [24] did not give clear signals on clustered spots in the pepscan analysis. Liautard *et al.* [31] reported four independent epitopes in another pannel of anti-IL-6R McABs, but did not identify the sequences of these antigenic determinants. We show here that aminoacid sequences corresponding to the linear epitopes recognized by McABs 34.4 and 50.6 function as antagonists of IL-6 action on growth of human myeloma cells and of murine hybridoma cells. Significant inhibitory activity was obtained at peptide concentrations of 50-100 μM, indicating a rather low affinity of the peptides for their target site. This suggests that the antagonist peptides act on sites at which receptor components may interact with low affinity (see below). Other peptides at similar concentrations had little or no effect. The antagonist peptides were able to inhibit an early signaling event mediated by the IL-6 receptor system such as the activation of Stat transcription factors to bind pIRE DNA elements [13, 14]. This activation involves tyrosine phosphorylations and requires the dimerization of the gp130 receptor component as a result of its interaction with IL-6 and IL-6R [10 - 12].

To get some insight on the position of the active peptide sequences within the IL-6R molecule and their potential target sites, we constructed a computer model of the receptor complex by assuming that the overall structure would follow the parameters established for the human growth hormone receptor by DeVos *et al.*

Table 5
Summary of the two proposed intractions between β-strands of the domain-ILs of human IL-6R and gp130

Interaction 1 gp130/Il-6R		Interaction 2 gp130/IL-6R		GHR interactions	
gp130	IL-6R	gp130	IL-6R	GHR-1	GHR-2
E	A-B, E	A (-B), G	C-C'	AB, E	AB, E

The β-strands designated as in DeVos et al [18] which contain interaction sites predicted by the model of Fig. 4 are indicated in comparison to the GHR model. The IL-6R peptide L^{255} - V^{270} (1063) and overlapping S^{247} - E^{260} (1062) would interfere with interaction 2.

[18]. The analogy between many cytokine receptors and the GHR has been stressed by Bazan [16] and, in the absence of crystallographic data for the IL-6 receptor complex, such modelization can give a rough prediction of the main interaction sites of IL-6R with other components of the receptor complex. The main interactions of IL-6R are expected to be with its IL-6 ligand and with the gp130 molecule, and these were also modeled in analogy to GH and GHR respectively. However, in variance from the GHR homodimer, the IL-6 receptor heteromeric complex must allow for the dimerization oftwo gp130 molecules. Recently, experimental evidence has been provided in favor of a hexameric structure of the IL-6 receptor system, consisting of two molecules each of IL-6, IL-6R and gp130 [19, 20]. The structure of the IL-6 receptor complex would, therefore, be a symmetrical assembly as shown schematically in Fig. 4 (part B), in which IL-6R would interact with two gp130 molecules and gp130 with two IL-6R.

The predicted 3-dimensional model of the hexamer is shown in Fig.4 (Part A) and is restricted to the cytokine domain of the human IL-6R and gp130, not including the Immunoglobulin-like domains at the N-termini of IL-6R and gp130, nor the Fibronectin-like domains of gp130. In this model, the sequence forming the core epitope of McAB 34.4 (peptide L^{255} - V^{270}, colored green-red-green in Fig. 4A) is not in the sites predicted to bind IL-6, but rather could be identified as one of the interaction sites between IL-6R and gp130. This sequence is in the domain-II of IL-6R at the junction of β-strands C and C' (or D, see refs 16, 18) and forms a protruding loop which comes in contact with gp130 (yellow in Fig. 4A). The peptide L^{255} - V^{270} may, therefore, interfere with the interaction of IL-6R and gp130 in their domain-II. This may also explain the action of the partly overlapping peptide S^{247} - E^{260}, which contains the exposed antigenic determinant of McAB 50.6 on strand C (grey and green, Fig. 4A). In the model, the interaction site between the second gp130 molecule and the domain-II of IL-6R is predicted to involve the opposite β-sheet namely the junction of strands A and B (corresponding to peptide A^{226} - N^{245}) and strand E of IL-6R (contained in peptide C^{277}-G^{299}). Mutations in residues H^{280}, D^{281} and also A^{228} and N^{230} were reported to affect the activity of IL-6R [20, Toniati C and Ciliberto G, private communication]. Peptide A^{226}-N^{245} (1086) had indeed some IL-6 antagonist activity in our experiments. In contrast, peptide C^{277} - G^{299} (containing H^{280} and D^{281}, i.e. peptide 983 in Table 1) had no significant activity and even the shorter peptide V^{290} - Q^{300} (1064) which contains a region of the IL-6R predicted to be binding the IL-6 ligand such as the RAQEEF sequence [32] did not exhibit antagonist activity.

These considerations suggest that some of the interactions between the domain-II of IL-6R and of gp130 may be of low enough affinity to allow peptides to interfere, whereas the interactions with ligand, which are of higher affinity, would be less amenable to inhibition by peptide analogs.The model proposed for the two interactions of the domain-II of IL-6R and of gp130 is summarized in Table 5. Interaction 1 has some homology to that in the GHR model, with the difference that the AB loop in gp130 is not involved. Interaction 2 has not been seen in the GHR model and seems to involve an exposed region (C-C' loop) which contributed the linear antigenic determinant for the McABs. Interaction 2 seems also to be the most sensitive to inhibition by exogenous peptides in our experiments. Other interactions, for example in the domain-I, remain to be investigated. In view of the position of the core epitope of McAB 34.4 (sequence L^{255} - V^{270} or peptide 1063) in the putative model of IL-6R, one may wonder why this McAB inhibits binding of IL-6 to the isolated sIL-6R and to cells [4, 24]. Possibly this inhibition is due to steric hindrance rather than to direct interaction with ligand binding. In addition, the solid phase assay of IL-6 binding to sIL-6R isolated from urine, may not be a true reflection of the interaction between IL-6 and the receptor hexameric complex as it occurs on the cell surface.The inhibition of IL-6 binding to cells by McAB 34.4 could be explained by inhibition of the formation of the IL-6R/gp130 complex since this interaction is needed for high affinity binding [2]. The epitope of McAB 50.6 is closer to a region implicated in IL-6 binding and steric hindrance is likely [21].

Our study is in partial agreement with the previous work of Grube and Cochrane [23] who showed that peptide Y^{249}-T^{264} was active (at 64 μM) to inhibit the effect of IL-6 on the growth of murine hybridoma B9 cells. This peptide was not assayed on a human myeloma, but instead the effect of IL-6 on fibrinogen

induction in human hepatoma HepG2 cells was measured. In this latter assay, Grube and Cochrane showed that peptides Y^{249} - T^{264} and Y^{249} - R^{258} were active, but that peptide L^{255} - W^{268} was not active. In our work on XG-1 and B9 cells, we find both peptides S^{247} - E^{260} (1062) and more so L^{255} - V^{270} (1063) to be active. Possibly the discrepancy about activity of peptide L^{255} -V^{270} stems from the two additional residues as compared to L^{255} - W^{268}, or the discrepancy is due to the assay system. In particular the stability of the peptides during the assay may be critical, and this may vary with their length, their ultimate aminoacid, as well as with the cell type used for the assay. Our work was done before Grube and Cochrane's publication [23], and we chose peptide L^{255} - V^{270} on the basis of the epitope mapping of McAB 34.4. The activity of this peptide to inhibit the IL-6 dependent growth of B9 cells and of XG-1 cells was verified in numerous experiments. Whether the adjacent peptides S^{247} - E^{260} (1062, epitope of McAB 50.6) and L^{255} - V^{270} (1063, epitope of McAb 34.4) act by the same or by different mechanism will be interesting to investigate. The model of action that we propose may also, hopefully, allow to develop peptide antagonists of IL-6 which act at much lower concentrations.

Although this discussion relates to the structures of the human IL-6R and gp130 molecules, it is likely that the same model explains the action of the eptide antagonists on the mouse hybridoma cells. According to the model (Fig. 4), the L^{255} - V^{270} and S^{247} - E^{260} peptides from human IL-6R would bind to regions of gp130 (yellow in Fig. 4) and the amino-acids in these regions are very conserved in the murine gp130 sequence [33]. In fact all residues making contacts with the above peptides were seen to be conserved in human and mouse gp130. The IL-6R sequences corresponding to the above peptides show also high homology in human and mouse IL-6R, in particular in the protruding loop (red in Fig. 4).

Understanding the function of the IL-6 receptor system is of importance in view of the potential medical applications of IL-6 in hematopoiesis and as an antimetastatic agent [34 - 36]. On the other hand, inhibitors of IL-6 may have applications in diseases such as multiple myeloma, some immune dysfunctions and postmenopausal osteoporosis [25, 37, 38]. Inhibition of IL-6 action *in vivo* and *in vitro* was obtained with anti-IL-6 monoclonal antibodies [39] or with muteins of IL-6 [29, 40]. Peptides or peptidomimetics which could interfere with the interaction between IL-6R (or the sIL-6R agonist) and gp130 within the receptor complex appear as an attractive alternative approach.

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A monoclonal antibody recognizing an epitope shared by receptors for growth hormone, prolactin, interleukin 2 and interleukin 6

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Abstract

Monoclonal antibody (MAb) termed R7B4 was generated throughout the idiotypic-anti-idiotypic network from mice immunized with human and bovine growth hormones (GH). The Ab was selected on the basis that it did not recognize human GH (hGH) neither insolubilized nor in solution but inhibited ^{125}I-hGH binding to receptors from rat and rabbit liver and from Nb2-cell membranes.

Since it inhibited Nb2-cell mitogenesis stimulated by hGH, prolactins or placental lactogens, MAb R7B4 behaved as an antagonist of lactogenic hormones. Furthermore, the Ab impaired proliferative activity of interleukin 2 (IL-2) on Nb2 cells as well as growth of 7TD1 cells, an interleukin 6 (IL-6) dependent hybridoma not expressing GH receptors.

Biotin-labeled MAb R7B4 specifically bound to rat liver microsomes, and the Ab was able to recognize Nb2 and 7TD1-cell membranes as shown by flow cytometry experiments. However, MAb binding was not hampered by hGH, indicating that the Ab did not mimic GH binding site to receptors. Immunoblot assays indicated that rat and rabbit liver as well as Nb2-cells membrane antigens recognized by MAb R7B4 were similar to those revealed by a MAb directed to prolactin receptors. In addition, MAb R7B4 was able to detect two bands probably corresponding to the somatogenic receptor in rabbit liver microsomes as well as three different proteins in 7TD1-cells showing molecular weights similar to those of the IL-6 receptor complex.

Results suggest that MAb R7B4 is directed to an epitope shared by receptors for lactogenic and somatogenic hormones, IL-2 and IL-6. To our knowledge, these data are the first experimental evidence of the existence of structural similarity between some of the receptors grouped in the cytokine receptor superfamily. (Mol Cell Biochem **195**: 235–243, 1999)

Key words: monoclonal antibody, receptors, lactogenic hormones, interleukin 2, interleukin 6

Introduction

Most of the receptors for hematopoietic growth factors share common structural motifs in the extracellular binding domain that allow them to be grouped into the cytokine receptor superfamily [1]. Various cytokines, such as erythropoiltin (EPO), growth hormones (GH), prolactins (PRL), thrombopoietin and granulocyte-specific colony-stimulating factor (G-CSF) utilize receptors consisting of a single chain [2]. The receptors for interleukin-6 (IL-6), IL-11, oncostatin M, leukaemia inhibitor factor (LIF) and ciliary neurotrophic factor (CNTF) all signal through a common β chain called gp130 [3]. Other cytokine receptors (IL-2, IL-4, IL-7, IL-9 and IL-15) utilize a shared γc, subunit and ligand specific α and/or β subunits [2, 3]. Structurally, those receptors are also related to the receptors for interferons (IFN), and it is proposed that they all evolved from a common progenitor [2].

In the search for anti-idiotypic monoclonal antibodies (MAb) mimicking the GH configuration recognized by specific receptors [4], we prepared hybridomas from mice

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immunized with a mixture of human (hGH) and bovine (bGH) growth hormones. A MAb, termed R7B4, unable to recognize GH while capable of inhibiting hGH binding to its different receptors, was isolated and characterized. Results presented thereafter indicate that MAb R7B4 impaired cell proliferation induced by lactogenic hormones through interaction to PRL receptors but also inhibited IL-2 and IL-6 mitogenic activity, suggesting that epitope R7B4 is involved in the various ligand/receptor interactions.

Materials and methods

Hormones and interleukins

Pituitary hGH (AFP-9755-A) and ovine prolactin (oPRL, AFP-9221A) were provided by the National Hormone and Pituitary Program, NIDDK, University of Maryland School of Medicine, Bethesda, MD, USA. Bovine GH (bGH) was prepared in our laboratory following the method of Dellacha and Sonenberg [5].

Recombinant mouse interleukin 6 (IL-6) was a generous gift of Drs. J. Van Snick and J-C. Renauld (Ludwig Institute, Brussels, Belgium). Recombinant human interferon α2b (IFNα2b) was provided by Biosidus S.A. (Buenos Aires, Argentina). Rat interleukin 2 (IL-2), human placental lactogen (hPL) and insulin from porcine pancreas were purchased from Sigma Chemical Co, St. Louis, MO, USA.

Radioiodination

^{125}I-hGH, ^{125}I-IFNα2b and ^{125}I-IL-6 were prepared following the method described by Roth [6]. Specific radioactivities ranging from 70–120 μCi/μg were usually achieved. Porcine insulin was iodinated as described by Balbis *et al.* [7]. Specific activity ranged from 150–200 μCi/μg.

Antibodies

The preparation and characterization of anti-hGH MAb 3C11 and AC3 have been reported elsewhere [8]. MAb T6, directed exclusively to PRL receptors, was acquired from Affinity BioReagents, Inc. (Golden, CO, USA). Control Ig was obtained from sera of nonimmunized BALB/c mice.

Production and screening of MAb

Three-month-old female BALB/c mice were immunized subcutaneously on days 1, 15, 30 and 45 with a mixture of 10 μg each of hGH and bGH emulsified in Freund's complete adjuvant (Gibco BRL, Gaithersburg, MD, USA). Three days before the fusion two mice were injected i.p. with 150 μl of phosphate-buffered saline (PBS) containing 25 μg of each hormone. The fusion procedure was performed according to the method of Galfre and Milstein [9] using NSO-myeloma cells as the fusion partner.

The reactivity of cell culture supermatants with either insolubilized hGH or bGH was tested by a solid phase radioimmunoassay (RIA). Polyvinyl microplates (Becton Dickinson, Oxnard, CA, USA) coated with each hormone (1 μg/ml diluted in 5 mM glycine buffer, pH 9.2) were incubated 4 h at 37°C with 200 μl of hybridoma supermatants. After washing with PBS containing 0.125 ml of Tween 20 per liter (PBS-Tween), the reacting Ab were revealed with goat ^{125}I-Ab to mouse Ig. Negative samples were immediately assayed for their ability to inhibit ^{125}I-hGH binding to rat liver microsomes as described below. In order to detect Ab binding solely to hormone in solution, supermatants showing no reactivity with insolubilized GH but inhibiting ^{125}I-hGH binding were incubated with ^{125}I-hGH and submitted to HPLC gel-filtration on a Bio-Sil TSK-250 column (Bio-Rad Laboratories, Richmond, CA, USA) as reported [8].

Hybridoma 7B4 was cloned twice by limiting dilution and supermatants were tested as it is described above. A positive clone, named R7B4, was selected and later amplified as ascitic tumors in BALB/c mice primed with 0.5 ml of pristane (2, 6, 10, 14-tetramethylpentadecane). The MAb was used either semipurified by precipitation with 50% (v/v) ammonium sulphate or purified by affinity chromatography as described below. Antibody containing supermatants from cloned cultures were isotyped using standard methods [9].

Liver microsomal preparations

Livers from late pregnant Wistar rats and female rabbits (New Zealand White, 2.5kg) were homogenized in 10 vol (v/w) of chilled 0.3 M sucrose, 5 mM Tris/HCl buffer, pH 7.4, and centrifuged at 12,000 × g for 20 min and then at 100,000 × g for 1 h. The pelleted microsomes were resuspended in 25 mM Tris/HCl buffer, pH 7.4 and frozen at –20°C until use. The protein concentration, determined by the method of Lowry *et al.* [10], was 60 mg/ml in both preparations.

Crude rat liver membrane preparations employed in Western Blot experiments were obtained after homogenizing tissues in 10 vol (v/w) of PBS containing 1 mM phenylmethylsulfonylfluoride (PMSF) and centrifuged at 10,000 × g for 15 min at 4°C.

Cell lines and cell proliferation assays

Suspension cultures of lactogen-dependent Nb2 lymphoma cells were maintained in Fischer's medium (Gibco BRL, Gaithersburg, MD, USA) containing 50 U/ml penicillin, 50 μg/ml streptomycin, 100 μM 2-mercaptoethanol (2-ME), 10% horse serum (HS) and 10% fetal calf serum (FCS). Cell

proliferation assays were carried out as described by Tanaka *et al.* [11]. Briefly, 24 h before their use in the assay, cells were transferred to Fischer's medium supplemented with 1% FCS, 10% HS, 100 μM 2-ME and antibiotics (low FCS Fischer's medium) and incubated for 24 h in order to slow down the rate at which the cells replicated. At the end of this period the cells were collected by centrifugation and re-suspended at a concentration of 7×10^4 cells/ml in Fischer's medium formulated as above but lacking FCS. A volume of 0.1 ml of cell suspension, containing 0.2 ng/ml of hGH or 10 U/ml of IL-2, was incubated in 96-well plates with the addition of the same volume of medium containing the sample to be tested. After 3 days of incubation, cell number was evaluated by colorimetric determination of hexosaminidase levels [12].

7TD1 cells, an IL-6-dependent hybridoma [13], were kindly provided by Drs. J. Van Snick and J-C. Renauld (Ludwig Institute, Brussels, Belgium). Cells were grown in Iscove's medium (Gibco BRL, Gaithersburg, MD, USA) supplemented with antibiotics, 10% FCS, 2 mM glutamine, 0.24 mM asparagine, 0.55 mM arginine, 50 μM 2-ME, 0.1 mM hypoxanthine, 16 μM thymidine and 200 U/ml of mouse IL-6. Before the assay, the cells were washed three times with medium without IL-6 and resuspended at 20,000 cells/ml in culture medium containing 100 U/ml of IL-6. A hundred microliters of this suspension was added to 96-wells microplates containing serial dilutions of samples (MAb or control Ab) in the same volume of medium lacking IL-6. After incubating 3 days at 37°C, the number of cells in each well was determined as indicated above [12].

WISH cell line (ATCC CCL 25), derived from human amnion tissue, was cultured in Minimun Essential Medium (MEM, Gibco BRL, Gaithersburg, MD, USA) supplemented with antibiotics, 10% FCS and 2 mM glutamine.

Cell-membrane preparations

Approximately $1–2 \times 10^8$ 7TD1, WISH or Nb2 cells proliferating in their respective mediums were centrifuged 10 min at 400 × g and homogenized in 10 ml of chilled binding medium (B.M.: 25 mM Tris/HCl, 10 mM $MgCl_2$, 0.1% BSA, pH 7.4). After centrifugation at 12,000 × g for 15 min the pellets were resuspended in B.M. at a membrane concentration equivalent to $1–4 \times 10^7$ cells/ml. A similar procedure was applied to prepare Nb2 as well as 7TD1-cell membranes used in immunoblotting assays, except that $MgCl_2$ and BSA were omitted.

Binding of hGH, insulin, IFNα2b and IL-6 to their specific receptors

^{125}I-hGH (0.5–2 ng) was incubated overnight at 25°C in B.M. (total volume: 0.3 ml) with either liver microsomes (rat: 30 μg of microsomal protein; rabbit: 120 μg of microsomal protein) or cell-membrane suspension (100 μl) and serial dilutions of competitors (unlabeled hGH or Mab). The reaction was stopped by the addition of 4 ml of ice cold B.M. and the membranes were sedimented by centrifugation at 800 × g for 25 min at 4°C. Bound radioactivity was measured in an automated gamma counter. ^{125}I-hGH non-specific binding was determined in the presence of 3 μg/ml of unlabeled hGH. Essentially the same procedure was followed to measure ^{125}I-IFNα2b binding to WISH-cell membranes and ^{125}I-insulin to rat liver microsomes, except that incubation of insulin with receptors was carried out at 4°C.

Binding of ^{125}I-IL-6 to 7TD1 cells was performed by incubating 3 h at room temperature the tracer (approximately 120,000 cpm) and competitors with 2×10^6 cells in a 300 μl final volume of RPMI 1640 medium (Gibco BRL, Gaithersburg, MD, USA) containing 3 mg/ml BSA and 0.02% sodium azide. The cells were layered on 0.6 ml of chilled 0.3 M sucrose and centrifuged for 5 min at 800 × g. The tips of the tubes, containing the cell pellets, were cut off and radioactivity was measured in an automatic gamma counter.

Preparation of purified Ab

MAb R7B4 and control Ig were purified by affinity chromatography on a protein A-Sepharose 4B column (Sigma Chem. Co., St. Louis, MO, USA). Elution of bound IgG was done with 0.1 M glycine-HCl pH 3.6 [14]. Fractions showing maximal absorbance values were pooled and stored at –20°C.

Biotin-labeled MAb was prepared by incubating 4 h at room temperature 10 μg of NHS-LC-Biotin (Pierce Chemical Co, Rockford, IL, USA) diluted in dimethyl sulphoxide with 100 μg of Ig in $NaHCO_3$ 0.1 M pH 8.0. The reaction was stopped by addition of 8 μl of NH_4Cl 1 M and following dialysis against PBS the final product was stocked at –20°C in 50% (v/v) glycerol.

Binding of biotin-labeled MAb R7B4 to rat liver microsomes

Microsomes from rat liver (10 mg of protein) were incubated overnight with 0.75 ml of rat nonimmune serum in 25 mM Tris/HCl, $CaCl_2$ 10 mM, BSA 0.1%, pH 7.4 (total volume: 3 ml). The membranes were sedimented by centrifugation at 800 × g for 25 min at 4°C, washed with the same buffer and resuspended.

Polyvinyl microplates were coated overnight at 4°C with 100 μl of microsomal suspension (1.2 mg/ml of protein), washed with PBS-Tween and blocked for 2 h at room temperature with PBS-BSA 1%. Following washing, biotin-

labeled MAb R7B4 and competitors diluted in 100 μl of PBS-BSA 1%, Tween 20 0.5 ml/l, were added. After 2 h at 25°C the plates were washed and the bound Ab was detected by addition of avidin-peroxidase and OPD (Sigma Chem. Co., St. Louis, MO, USA).

Flow cytometry

Approximately 5×10^5 Nb2 or 7TD1 cells were centrifugated and resuspended in 150 μl of Fischer's or Iscove's culture medium, respectively, containing BSA 1%, sodium azide 0.1% and 10 μl of either MAb R7B4 or MAb AC3 ascitic fluid. After incubating 2.5 h at 4°C the cells were washed and resuspended in 100 μl of fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG (Dako A/S, Denmark) diluted 1/16 in the media indicated above. One hour later the cells were washed and examined by fluorescence on a Becton Dickinson FACScan.

Immunoblotting

Crude rat liver membrane preparations, cell membranes or microsomes from rabbit liver (approximately 100 μg of protein) were subjected to 10% SDS-PAGE [15] and transferred onto nitrocellulose sheets (Amersham, Buckinghamshire, UK). After completion of the electrophoretic protein transfer, non-specific antibody-binding sites on the nitrocellulose were blocked by incubation for 1 h at room temperature with 5% nonfat milk in PBS containing 0.1% Tween 20 (PBS-T). After washing four times with PBS-T the strips were incubated overnight at room temperature with either MAb R7B4, T6, or 3C11 diluted in PBS-T, 1% BSA. Following several washes with PBS-T, bound antibodies were revealed with peroxidase labeled donkey IgG anti-mouse IgG (Jackson Immunoresearch Laboratories, Inc., West Grove, PA, USA) and ECL reagents (Amersham, Buckinghamshire, UK). Molecular weight markers were from BDH Laboratory Supplies Poole BH15 1TD (UK).

Results

Hybridoma production and selection

Mice were immunized with a mixture of hGH and bGH according to the procedure described by Hill and Erlanger to obtain anti-idiotype Ab [4]. Since the aim of our work was to prepare Ab to GH-receptors we first discarded the hybridomas secreting Ab able to recognize either hGH or bGH insolubilized on a plastic surface. Even though four hybridomas showed not to react with hormones but to inhibit ^{125}I-hGH binding to rat liver receptors, gel filtration HPLC experiments demonstrated that only Ab secreted by hybridoma 7B4 did not bind the tracer. Thus, 7B4 cells were cloned and subsequently expanded as ascitic fluid to obtain a MAb that was termed R7B4. Isotype assays indicated that MAb R7B4 pertained to the IgG1 subclass.

Effect of MAb R7B4 on ^{125}I-hGH binding to receptors

As reported previously [16–17], receptors from rat liver and Nb2 cells display lactogenic specificity. Under our experimental conditions, half-maximal ^{125}I-hGH binding inhibition was obtained in the presence of $3–6 \times 10^{-10}$ M hGH, whereas bGH scarcely affected hGH binding. In order to saturate the lactogenic receptors also present in rabbit liver microsomes, the somatogenic specificity was achieved by adding oPRL 4.5 nM to the binding medium [17]. Thus, in the presence of oPRL, 50% ^{125}I-hGH binding inhibition was shown by either hGH or bGH at a concentration 7×10^{-10} M.

Results from Fig. 1 show that MAb R7B4 fully inhibited ^{125}I-hGH binding to lactogenic receptors from rat liver microsomes and Nb2 cells as well as hormone binding to somatogenic receptors from rabbit liver. Fifty percent of ^{125}I-hGH binding inhibition to rat liver receptors was obtained in the presence of 4×10^{-8} M Ab, whereas a concentration 2×10^{-8} M was required to attain the same effect on hormone binding to Nb2 cells or rabbit liver membranes (Fig. 1).

MAb R7B4 binding to liver microsomes

Biotin-labeled MAb was incubated with microsomes from rat liver and the bound Ab was revealed by the addition of avidin-peroxidase. Results showed that MAb R7B4 inhibited its own binding whereas control Ig did not produce any effect (Fig. 2). Surprisingly, MAb binding to microsomes was not affected by hGH. Optical density in the presence of 50 μg/ml of hormone was 1.130 ± 0.140, a value similar to those displayed by control Ab (Fig. 2).

Proliferation of Nb2 and 7TD1 cell lines

Nb2 cells were incubated with different concentrations of the MAb alone or in the presence of antiserum to mouse Ig. Results indicated that MAb R7B4 failed to induce Nb2-cell proliferation regardless the Ab concentration used or the addition of anti-mouse Ig (data not shown).

Since Nb2-cell mitogenesis could be induced by either hGH or IL-2, we tested the effect of MAb R7B4 under both conditions. Results indicated that cell proliferation was inhibited proportionally to the MAb concentration no matter what mitogen was used, whereas an irrelevant Ab did not

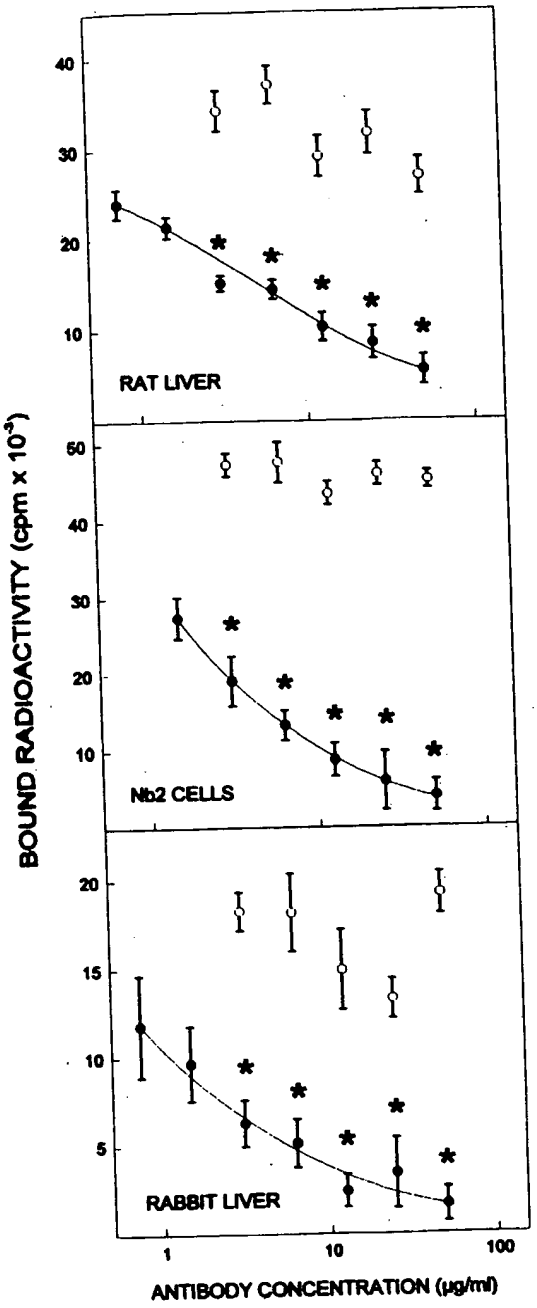

Figure 1. Effect of MAb R7B4 on ^{125}I-hGH binding to receptors from rat and rabbit liver and Nb2-cell membranes. The tracer (150,000–200,000 cpm) was incubated overnight at 25°C with rat liver microsomes (30 μg of protein), Nb2-cell membrane suspension (equivalent to 4 x 10^6 cells) or rabbit liver microsomes (120 μg of protein) in the presence of different concentrations of MAb R7B4 (solid symbols) or control Ig (open symbols). Bound radioactivity was measured after washing the membranes by centrifugation. ^{125}I-hGH nonspecific binding was determined in the presence of 3 μg/ml of unlabeled hGH. Values in the absence of inhibitor were: rat liver: 27,300 ± 550 cpm; Nb2 cells: 41,960 ± 2,100 cpm; rabbit liver: 14,500 ± 2,280 cpm. Results are means of three determinations and are expressed as ^{125}I-hGH specific binding ± S.D. Statistical significance in comparison with control value by Student's *t*-test is indicated by *($p < 0.005$).

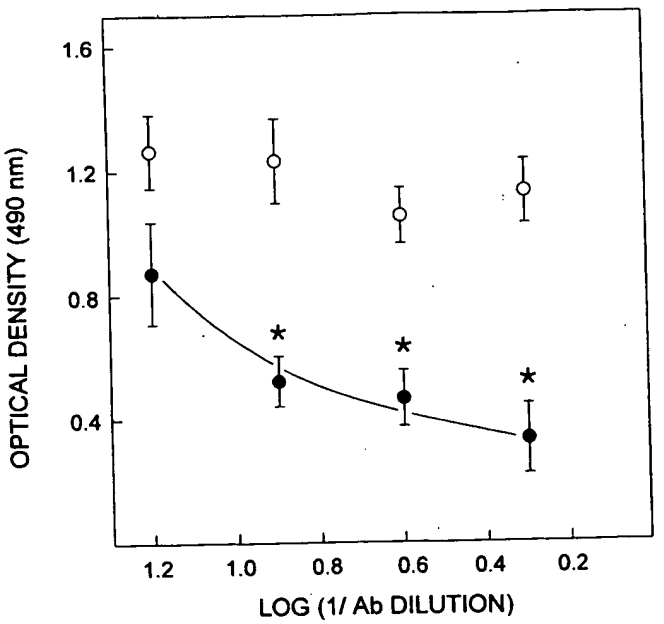

Figure 2. MAb R7B4 binding to rat liver microsomes. Biotin-labeled MAb R7B4 (0.1 μg) and competitors were added to microplates coated with rat liver membranes previously incubated with rat non-immune serum. After 2 h at 25°C the plates were washed and the bound Ab was detected by the addition of avidin-peroxidase and OPD. Solid symbols: MAb R7B4 ascitic fluid. Open symbols: ascitic fluid containing control Ig. Optical density in the absence of competitors was 1.090 ± 0.260. Results are means of three determinations ± S.D. Statistical significance in comparison with control value by Student's *t*-test is indicated by *($p < 0.005$).

produce any effect (Fig. 3). Analogous MAb R7B4 behavior was observed when Nb2-cell proliferation was stimulated with other lactogenic hormones such as oPRL or hPL instead of hGH (data not shown).

7TD1 is an IL-6-dependent hybridoma which has been widely characterized [13, 18]. ^{125}I-hGH-binding experiments demonstrated that 7TD1 cells do not express hGH receptors, because values of bound radioactivity were 24,200 ± 550 cpm and 23,800 ± 250 cpm in the absence and in the presence of 10 μg/ml of hGH, respectively. Concurrently, 7TD1-cell proliferation was not stimulated by hGH (data not shown).

Despite the absence of GH receptors, MAb R7B4 inhibited 7TD1-cell proliferation whereas cell number did not decrease in the presence of control Ab (Fig. 4).

MAb effect on binding of insulin, IFN and IL-6 to their specific receptors

In order to determine the specificity of MAb R7B4 binding inhibitory activity, we tested its effect on ^{125}I-insulin ^{125}I-IFNα2b and ^{125}I-IL-6 binding to rat liver, WISH-cell and 7TD1-cell receptors, respectively. Results showed that the Ab

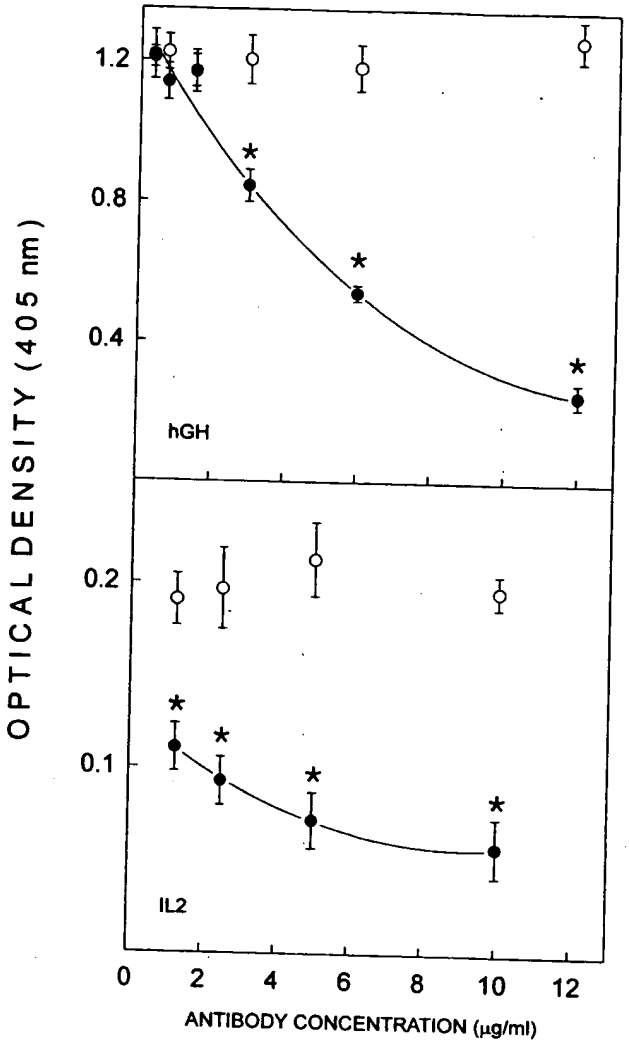

Figure 3. Effect of MAb R7B4 on Nb2-cell proliferation. Approximately 35,000 cells/ml were incubated for 3 days in the presence of 0.1 ng/ml of hGH (upper panel) or 5 U/ml of IL-2 (lower panel) and different concentrations of MAb R7B4 (solid symbols) or control Ig (open symbols). Cell number was determined by the hexosaminidase method [12]. Results are means of three determinations ± S.D. Optical density values in the absence of competitors were 1.210 ± 0.120 for cells incubated with hGH and 0.220 ± 0.020 for cells incubated with IL-2. Statistical significance in comparison with control value by Student's *t*-test is indicated by *($p < 0.005$).

did not affect neither insulin nor IFNα2b binding to their specific receptors even at a concentration that strongly inhibited ^{125}I-hGH binding, whereas a manifest MAb inhibitory effect on IL-6 binding was observed (Table 1).

MAb R7B4 binding to Nb2 and 7TD1 cells

Flow cytometry analysis showed that MAb R7B4 did bind to Nb2 and 7TD1 cells (Fig. 5). As shown previously for biotin-

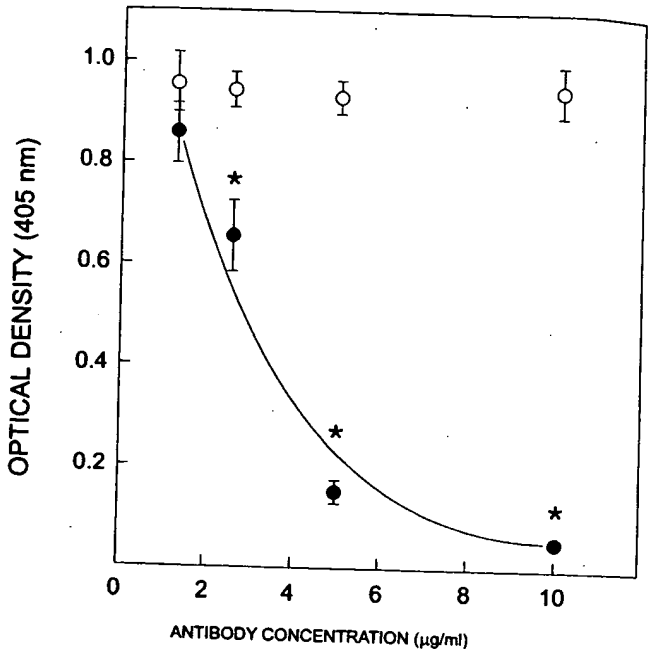

Figure 4. Effect of MAb R7B4 on 7TD1-cell line proliferation. Approximately 10,000 cells/ml were incubated for 3 days with 50 U/ml of IL-6 in the presence of different concentrations of MAb R7B4 (solid symbols) or control Ig (open symbols). Cell number was determined by the hexosaminidase method [12]. Results are means of three determinations ± S.D. Optical density in the absence of competitors was 0.940 ± 0.070. Statistical significance in comparison with control value by Student's *t*-test is indicated by *($p < 0.005$).

Table 1. Effect of MAb R7B4 on insulin, IFNα2b and IL-6 binding to receptors

Insulin (µg/ml)	IFNα2b (µg/ml)	IL-6 (µg/m1)	MAb R7B4 (µg/m1)	Bound radioactivity (cpm)
I)[a]				
–	–	–	–	7,200 ± 260
15	–	–	–	4,200 ± 130
–	–	–	50	7,050 ± 980
II)[b]				
–	–	–	–	35,400 ± 890
–	–	5	–	18,600 ± 950
–	–	–	50	35,650 ± 280
III)[c]				
–	–	–	–	3,400 ± 300
–	–	5	–	750 ± 100
–	–	–	10	1,850 ± 50

a)^{125}I-insulin (83,000 cpm) was incubated overnight at 4°C with rat liver microsomes (30 µg of microsomal protein) in the presence of either insulin or MAb R7B4 at the indicated concentrations. Results are expressed as bound radioactivity and are means of three determinations ± S.D. b)^{125}I-IFNα2b (260,000 cpm) was incubated overnight at 25°C with WISH-cell membranes (equivalent to 1 x 10^6 cells) in the presence of either IFNα2b or MAb R7B4 at the indicated concentrations. Results are expressed as bound radioactivity and are means of three determinations ± S.D. c)^{125}I-IL-6 (120,000 cpm) was incubated 3 h at room temperature with 2 x 10^6 7TD1-cells in the presence of either IL-6 or MAb R7B4 at the indicated concentrations. Results are expressed as bound radioactivity and are means of three determinations ± S.D.

labeled MAb binding to liver membranes, the presence of hGH did not impair MAb R7B4 binding to Nb2 cells (Fig. 5).

Immunoblotting assays

Crude membrane preparations from rat liver, Nb2 and 7TD1 cells and microsomes from rabbit liver were subjected to immunoblot analysis using MAb R7B4, 3C11 (anti-hGH) and T6 (anti-PRL receptor). As shown in Fig. 6, the pattern of reactivity of MAb R7B4 was identical to that of MAb T6 when Nb2-cell membranes were used. Likewise, in rat liver preparation both MAb detected five major bands, although two bands of mol wt 75,300 and 54,800, respectively, were revealed only in the presence of MAb T6. MAb 3C11, used as negative control, did not display any reactivity (Fig. 6).

As described above, microsomes from rabbit liver exhibit both somatogenic and lactogenic receptors. Accordingly, MAb R7B4 and T6 exposed two bands of mol wt 53,400 and 42,800, whereas two other antigens showing mol wt 140,000 and 68,500 were revealed exclusively with MAb R7B4 (Fig. 6). At least the major one of these latter bands would correspond to the somatogenic receptor, since it has been shown that receptors to somatotropins were associated with species of apparent molecular masses higher than 100 kDa [19].

Three major bands of mol wt 133,000, 76,500 and 66,700 were disclosed by MAb R7B4 in 7TD1-cell membranes subjected to SDS-PAGE and immunoblotting, whereas MAb 3C11 did not react at all (Fig. 6).

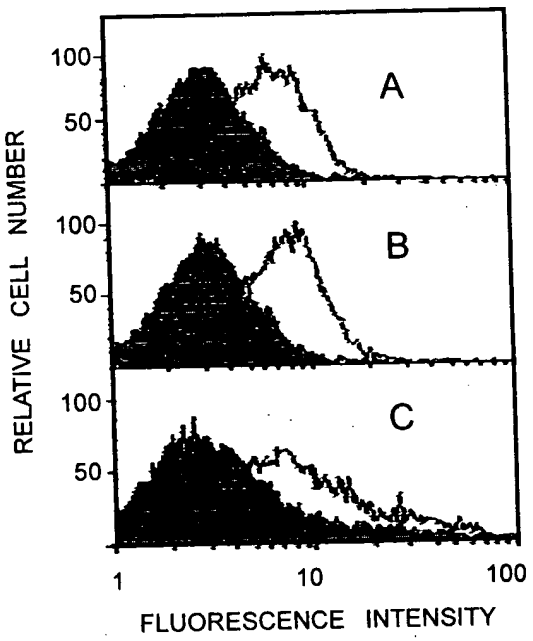

Figure 5. MAb R7B4 binding to Nb2 and 7TD1 cells determined by flow cytometry. Approximately 5 x 10^5 cells were centrifugated and resuspended in 150 μl of culture medium containing 10 μl of each ascitic fluid. After incubating 2.5 h at 4°C the cells were washed and incubated with FITC-conjugated goat anti-mouse IgG. After one hour the cells were washed and examined by fluorescence on a Becton Dickinson FACScan. Panel A: Nb2 cells incubated with MAb R7B4; panel B, Nb2 cells incubated with MAb R7B4 plus 65 μg/ml of hGH; panel C: 7TD1 cells incubated with MAb R7B4. In all cases controls were cells incubated with anti-hGH MAb AC3. Five thousand gated events were analyzed per sample. Mean gates ± S.D. were as follows: (A) control: 26 ± 13; MAb R7B4: 51 ± 14; (B) control: 26 ± 15; MAb R7B4 + hGH; 56 ± 15; (C) control: 30 ± 18; MAb R7B4: 49 ± 22. The results represented are derived from one of three independently performed experiments.

Discussion

It is well documented that certain antiidiotypic Ab are able to mimic biological properties of hormones, acting as internal images. Others act as antagonists of hormones, blocking their binding to receptor [20]. Antiidiotypic Ab can be induced through immunization with hormone or with antihormone Ab and are believed to arise as a consequence of idiotypic network interactions [21].

MAb R7B4 was obtained from hybridomas prepared from spleen cells of mice immunized with a mixture of hGH and bGH. To isolate a MAb simulating the GH site that interacts with receptors, a double screening was done. Once Ab recognizing either hGH or bGH were discarded, the ability of hybridoma supernatants to inhibit ^{125}I-hGH binding to receptors from female rat liver was determined. This procedure allowed us to isolate a MAb not recognizing hGH but capable of impairing hormone binding to both lactogenic and somatogenic receptors. Furthermore, the MAb behaved as a definite antagonist of lactogenic horinones, because it strongly inhibited Nb2-cell proliferation stimulated by hGH, oPRL or hPL.

MAb R7B4 bound to microsomes from rat liver as well as to Nb2 cells. However, simultaneous incubation with hGH did not hamper MAb binding, indicating that the Ab did not mimic GH binding site.

Because it was proposed that receptors for GH and PRL could have some structural similarity with receptors for several interleukins [1, 22], we tested the effect of MAb R7B4 on the IL-2-induced proliferation of Nb2 cells and the growth of the IL-6-dependent 7TD1 cell line, a mouse hybridoma not expressing hGH receptors. The MAb inhibited the mitogenesis of both cell lines in a dose dependent way, suggesting that epitope R7B4 is shared by IL-2 and IL-6 receptors and that its blockage impairs ligand activity. Accordingly, IL-6 binding was inhibited in the presence of MAb and flow cytometry data showed that 7TD1 cells did express R7B4 epitope. Furthermore, lack of MAb effect on insulin and IFNα2b binding to their receptors suggests Ab fairly restricted specificity.

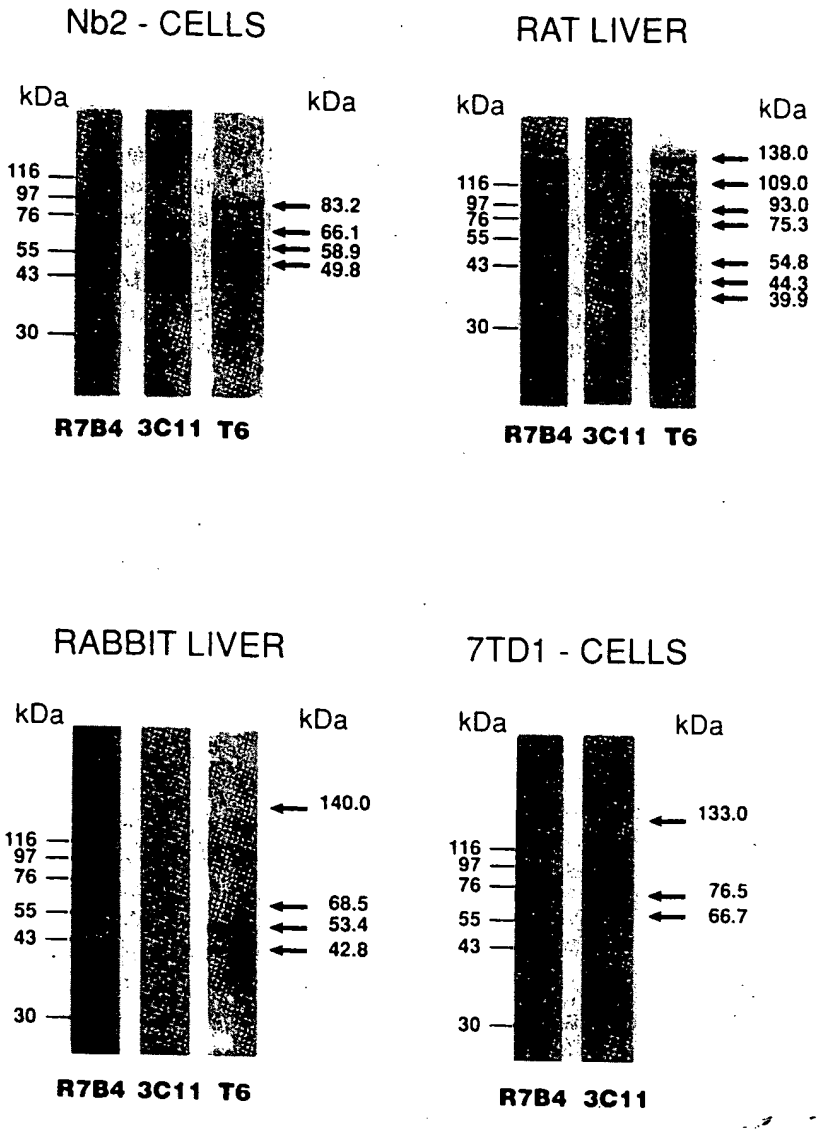

Figure 6. Reactivity of MAb R7B4, 3C11 and T6 with crude membrane preparations from rat liver, Nb2 and 7TD1 cells, and with microsomes from rabbit liver. Each sample (approximately 100 μg protein) was run in SDS-PAGE 10%, transferred onto nitrocellulose sheets and incubated with ammonium sulfate precipitated MAb R7B4 or 3C11 (50 μg/ml) or with protein A purified MAb T6 (5 μg/ml). The positions of molecular mass markers are shown at left.

Immunoblotting experiments performed with crude extracts of rat liver or Nb2 cells indicated that MAb R7B4 recognized essentially the same membrane proteins than did MAb T6, directed to PRL receptor [23]. Under our experimental conditions, MAb T6 showed to distinguish more bands than those previously reported [23]. Differences may be ascribed either to the detection method utilized in this work or to the source of material. In fact, ECL reagents does not require a labeled Ab, a procedure used when MAb T6 characterization was reported, and we examined crude tissue and cell extracts instead of isolated membranes to reveal the antigens defined by MAb R7B4 [23]. However, in spite of their similar reactivity towards immunoblotted proteins, MAb R7B4 and T6 behavior indicated that they are directed to different PRL receptor epitopes. Thus, MAb T6 and hGH competed for the same binding site [23] and the Ab exhibited mitogenic activity in Nb2 cells [24], whereas MAb R7B4 did not show these properties.

Correspondingly with its capacity of inhibiting hGH binding to rabbit liver microsomes and IL-6 binding to 7TD1 cells, MAb R7B4 recognized several species of molecular masses closed to those reported for somatogenic receptor [19] and IL-6 receptor complex [2], even though positive controls are needed to certify this fact. By the same token, MAb R7B4 identified a protein of mol wt 66,100 in immunoblotted Nb2-cell membranes. This mol wt can match with either one

of the several species of the PRL receptor, with the γ_c subunit of IL-2 receptor, or both [2, 23].

We are not able to explain the production of MAb R7B4 in terms of the idiotypic-antiidiotypic internal network, since the Ab is directed to receptors but it does not simulate the binding site of the antigens used to immunize the animals [4, 21]. More likely, MAb R7B4 seems to recognize an epitope required for binding of hGH, PRL, IL-2 and IL-6 to their specific receptors. Since receptors for these cytokines share structural motifs in their ligand binding domain [1] it is conceivable to hypothesize that epitope R7B4 arose from a similar folding of the various proteins. Thus, MAb R7B4 cross-reactivity should be the first experimental evidence of a common epitope allocated in somatogenic and lactogenic receptors as well as in receptors for IL-2 and IL-6.

Acknowledgements

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Concise Review

Immunotherapy of Multiple Myeloma

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Key Words. Immunotherapy • Multiple myeloma • Antibodies • Interleukins • Bone marrow

Abstract. In 1994, an estimated 12,700 new cases of multiple myeloma (MM) will be diagnosed in the USA and 9,800 patients will die from this disease. At present, a cure for MM has not been achieved with any chemotherapeutic regimen. Therefore, it is important to develop novel therapeutic approaches to treat this fatal disease.

This review focuses on new concepts in the immunotherapy of MM. Thus far, interferons and anti-human interleukin (IL)-6 monoclonal antibodies (MAbs) have been used to treat patients with this disease. Bone marrow transplantation using autologous marrow purged with MAbs and complement, with anti-myeloma immunotoxins (ITs), or MAb-magnetic bead conjugates has been reported. Adoptive cellular therapy, in vivo with anti-CD3 and IL-2, as well as transplantation of purified autologous CD34$^+$ peripheral blood stem cells, is now being evaluated in clinical trials. Anti-human IL-6 receptor (IL-6R) and anti-CD54 (ICAM-1) MAbs have shown promising results in the therapy of human myeloma cell lines in SCID mice, while an IL-6 antagonist protein, anti-gp130 MAbs, recombinant soluble gp130, anti-B7, anti-HLA-DR, and recombinant soluble CD16 also inhibit the growth of myeloma cell lines in vitro. These experimental therapeutic modalities hold promise for use in humans and may also provide further insights into the pathogenesis of MM.

Introduction

Multiple myeloma (MM) is a hematologic malignancy resulting from the uncontrolled proliferation of a single clone of plasma cells in the bone marrow (BM). The major clinical features of the disease include monoclonal gammopathy, lytic bone lesions, hypercalcemia, renal failure, anemia and immunodeficiency [1-3]. In 1994, an estimated 12,700 new cases will be diagnosed in the United States and 9,800 patients will die from this disease [4]. The disease occurs predominantly in elderly individuals with a median age at diagnosis of 70 years. Fewer than 15% of MM patients are under the age of 50 [5]. The overall prognosis for patients with MM has improved only slightly since the introduction of chemotherapy three decades ago. The overall median survival time is still approximately three years, and five-year survival rates occur in about 25% of treated patients [5, 6]. Standard therapy (melphalan and prednisone) induces responses in 50% of treated patients, but only 5% achieve complete remissions (CRs) [7]. Intensive combination chemotherapy induces a higher response rate (72%) and is effective for patients who are resistant to standard therapy. However, survival rates have not improved [6-8]. To date, clinical long-term remissions in MM patients have not been achieved with any chemotherapeutic regimen. Thus, several new therapeutic approaches are under development. Interferon-alpha (IFN-α) prolongs survival when combined with chemotherapy or when used as maintenance therapy [9, 10]. Encouraging results have been reported in patients using high-dose chemotherapy, with or without total body irradiation, followed by transplantation with syngeneic, allogeneic or autologous BM, or autologous peripheral blood stem cells (PBSCs). These approaches have shown a higher CR rate (ranging from 25%-80%) and longer progression-free survival [11]. However, these approaches have significant limitations, particularly for allogeneic marrow rescue. These include age, the lack of an HLA-compatible donor, the risk of graft versus

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host disease and treatment-related mortality (30%) [12, 13]. Autologous marrow or PBSC transplantation is possible for most patients, but successes have been hampered by the contamination of autografts with tumor cells or their precursors, and these cells may be responsible for relapse [13-16]. Many patients succumb to their malignancies soon after transplantation due to recurrence of disease [17].

Over the past few years, substantial progress has been made in our understanding of the pathogenesis of MM. It is now known that many cytokines play important roles in the development and progression of MM. Among these, interleukin (IL)-6 plays a major role, although other cytokines such as IL-3, IL-5, IL-8, IL-11, granulocyte-macrophage colony-stimulating factor (GM-CSF), leukemia inhibitory factor (LIF) and oncostatin M (OSM) have also been implicated in regulating the growth of MM [18-21]. A number of studies have demonstrated that IL-6 is a major autocrine and paracrine growth factor for human myeloma cells [22, 23]. Elevated serum levels of IL-6 are also responsible for accompanying manifestations of this disease such as anemia, thrombocytopenia, elevated acute phase proteins and myeloma-associated bone absorption [24, 25]. The elevated serum levels of IL-6 in MM patients correlate with both stage and the severity of the disease [26]. Although myeloma cells can produce IL-6, recent studies have suggested that BM stromal cells might be a more important source of IL-6 [27, 28]. Hence, BM stromal cells from myeloma patients can secrete significant amounts of IL-6 which increase during the adhesion of myeloma cells [27, 28]. *Zhang et al.* [29] have recently demonstrated that the growth of myeloma cells from 25 of 26 patients with advanced or terminal disease was dependent upon the presence of IL-6 in vitro. Development of transplantable monoclonal plasmacytomas with t(12:15) chromosomal translocation in IL-6 transgenic BLAB/c mice has further confirmed the critical role of IL-6 in this disease [30]. Therefore, therapeutic strategies directed at influencing the levels and activity of IL-6 may be clinically important.

The availability of new antibodies (Abs) and molecular probes, as well as the development of high-resolution flow cytometry and more sensitive and specific polymerase chain reaction (PCR)-based technologies, have made it possible to detect minimal residual disease and to more definitively identify circulating myeloma cell precursors [15, 16, 31]. Several studies have suggested that circulating monoclonal B lymphocytes may be the precursors of myeloma cells [32, 33] since these cells express the same idiotype and plasma cell-associated antigens as the myeloma cells in the BM [34]. More importantly, in the presence of IL-3 and IL-6, the peripheral blood mononuclear cells (PBMCs) from myeloma patients develop into plasma cells with the same isotypes that are expressed on the myeloma cells [35]. In vivo dissemination and growth of MM may involve interactions between the monoclonal B cell clone (myeloma precursor) and the BM stroma. The malignant B cell clones express adhesion molecules enabling the cells to bind to BM stromal cells and can release cytokines, such as IL-1β and GM-CSF. These cytokines induce BM stromal cells to produce other cytokines such as IL-6, IL-8 and IL-11. IL-6 and IL-11 may, in turn, stimulate the differentiation and proliferation of myeloma cells and their precursors [19-21]. How these circulating malignant B cells influence the success or failure of therapy is poorly understood. A better understanding of the nature of these circulating myeloma cell precursors may lead to more effective therapies for the disease.

Several human SCID/MM cell line models have recently been established [36, 37]. These animal models should facilitate the preclinical evaluation of novel immunotherapeutic strategies and increase our understanding of the pathogenesis of MM.

In this article, we will focus on new concepts in the development of immunotherapeutic approaches for MM and their potential applications.

Rationale for Immunotherapy in MM

Given our increasing knowledge of the human immune system and the biology of MM, it should be possible to design more specific immunotherapeutic strategies for this disease. These strategies can be divided into approaches in which the goal is to kill tumor cells by targeting via tumor cell surface molecules or in which the goal is general augmentation or restoration of the suppressed immune response. Although MAbs specific for malignant plasma cells have not yet been defined, several MAbs which recognize plasma cell-associated and/or differentiation antigens have been described

[38-41]. These MAbs may inhibit tumor growth by neutralizing critical growth factors [42], directly blocking growth factor receptors [43, 44], interfering with growth factor signal transduction [20, 45, 46], triggering apoptosis or cell cycle arrest [47] or mediating in vivo effector functions [48]. MAbs coupled to toxins (immunotoxins [ITs]), anti-cancer drugs or free-radical-producing enzymes, may kill tumor cells and spare normal cells. In addition, some growth factor-toxin fusion proteins can also be used to kill tumor cells which overexpress growth factor receptors [49, 50]. Therefore, these agents may be very useful in clearing tumor cells from the BM prior to autologous BM transplantation (ABMT) and/or in systemic therapy [51-54].

Tumor-induced immune suppression and tumor escape mechanisms are also important in the pathogenesis of MM. Several approaches have been developed to overcome disease-associated immunosuppression in order to restore and possibly augment anti-tumor immunity. These approaches include the use of IL-2, interferons (IFNs), and anti-CD3 MAbs as well as adoptive therapy with lymphokine-activated killer (LAK) cells or natural killer (NK) cells [55-57].

Targeting Human IL-6 and its Receptor

Due to the importance of IL-6 in the pathogenesis of MM, many different therapeutic strategies have been explored with the aim of interfering with IL-6 production and signaling (Table I).

Interference with ligand-receptor interaction by anti-IL-6 or anti-IL-6R MAbs, mutated IL-6 protein and the IL-6 antagonist, suramin, has been reported. Several anti-human IL-6R MAbs inhibit the proliferation of human myeloma cells in vitro and the growth of myeloma cells and cell lines in SCID mice [44, 58, 59]. *Taetle et al.* [43] have recently demonstrated that combined therapies using anti-transferrin receptor (TR) MAbs and anti-IL-6R MAbs have synergistic inhibitory effects on the growth of human myeloma cell lines in vitro. Some cell lines which do not respond to anti-IL-6R MAbs are inhibited by anti-TR MAbs. *Sato et al.* [59] have also humanized the murine anti-human IL-6R MAb, PM-1. In humans, anti-IL-6 MAbs have been used to treat refractory MM. In five of nine patients, myeloma cell proliferation was decreased in vivo, and the symptoms of the disease were improved. However, the effect was transient, since human anti-mouse-Ig (HAMA) and circulating monomeric IL-6/anti-IL-6 immune complexes were eventually produced [42, 60, 61]. The only side effect of anti-IL-6 MAbs was partial reduction (35-86%) in platelets in four of five patients receiving anti-IL-6 MAb for more than one week, without necessitating platelet transfusion [62]. It was unclear, however, whether the anti-IL-6 MAb had been injected in sufficient amounts to block IL-6 bioactivity in vivo [63].

In the Netherlands, a phase I/II study in refractory MM patients has been initiated, using a humanized MAb against IL-6 [64]. Preliminary results indicate that this chimeric anti-IL-6 MAb is well tolerated. Serum C-reactive protein levels (IL-6-dependent) were suppressed in all five patients, and in two of five patients the in vivo BrdU labeling index (LI) returned to normal levels during infusion. However, in three other patients, LIs were elevated. There was no evidence for the production of antibodies against the human MAb.

Using neutralizing MAbs to epitope map human IL-6, *Brakenhoff et al.* [65] have identified two distinct functional sites on the IL-6 molecule. Site I is a receptor-binding site, and site II is involved in the activation of the signal transducer, gp130. By using site-directed mutagenesis, they have constructed a recombinant

Table I. Potential therapeutics targeting IL-6 and its receptor

IL-6 Competitors
MAbs to IL-6 or CD126
mutated IL-6 proteins
IL-6-toxin fusion proteins
suramin
Interruption of IL-6 Signal Transduction Pathway
MAbs to CD130
recombinant soluble CD130 (sCD130)
anti-CD126
Inhibitors of IL-6 Production
IL-4
IL-1 receptor antagonists
antisense oligonucleotides
corticosteroids
non-steroid anti-inflammatory agents
Inhibitors of CD126 Expression
retinoic acid
IFN-γ

site II-mutated IL-6 protein (with a double substitution of Gln-160 with Glu and Thr-163 with Pro) which binds to the IL-6R with high affinity but does not transduce a signal because it cannot associate with gp130. Therefore, it inhibits the biological activities of IL-6. This IL-6 antagonist may be useful for treating patients with IL-6-dependent MM [66]. This antagonist should have little immunogenicity and might be useful for repeated courses of therapy.

Elimination of tumor cells expressing the IL-6R with an IL-6-toxin fusion protein is another attractive therapeutic approach. Several genetically engineered IL-6-toxin fusion proteins consisting of the receptor-binding moiety of the IL-6 molecule and the enzymatic fragments of bacterial toxins (devoid of their native cell recognition domain) such as mutated *Pseudomonas* exotoxin (PE40) [49] or diphtheria toxin (DAB389) [50] have been developed. These chimeric toxins bind to the IL-6R on tumor cells and kill them. Both the IL-6-PE40 and DAB389-IL-6 fusion proteins kill myeloma cell lines and fresh myeloma cells from patients in vitro and do not affect normal BM hematopoietic progenitors [49, 67]. These highly potent IL-6-toxin fusion proteins have entered clinical trials. However, targeting IL-6R$^+$ cells with chimeric toxins presents a potential problem since many normal cells, such as hepatocytes, constitutively express the IL-6R in vivo [62]. It has, therefore, been suggested that these chimeric toxins would be more suitable for ex vivo purging of BM from MM patients [49].

The IL-6R complex is composed of two distinct chains. Binding of IL-6 to the IL-6R (gp80, CD126) triggers the association of the IL-6R and a non-IL-6 binding signal transducer gp130 (CD130), forming a high-affinity IL-6 binding site. CD130 then transduces a signal into the cell [68]. Soluble (s)CD126, which lacks the transmembrane and intracellular domains, has been found in human serum and urine. In the presence of IL-6, sCD126 associates with membrane-anchored CD130 and stimulates sensitive cells [69]. Although sCD130 is also found in vivo, it behaves as an antagonist which can interact with the IL-6/CD126 complex but not transduce a stimulatory signal since it lacks the transmembrane and cytoplasmic portions of the molecule [68]. The requirement of CD130 activity for IL-6 signaling suggests that CD130 may be a potential target for anti-tumor therapy. In this regard, anti-CD130 or recombinant sCD130 is being explored for its ability to interfere with signaling via CD130. *Taga et al.* [45] have shown that anti-CD130 inhibits IL-6 signaling by preventing the physical association of CD130 and CD126, thereby suppressing the biological activity of IL-6 in vitro [46]. The MAbs also abrogate the proliferative activity of LIF, ciliary neurotrophic factor (CNTF), OSM and IL-11 on myeloma cells because the receptors for these cytokines share the CD130 subunit [20, 21, 46]. *Nishimoto et al.* [20] and *Zhang et al.* [21] have demonstrated that LIF, OSM, IL-11 and CNTF also stimulate the growth of freshly isolated plasmacytoma cells and that anti-CD130 MAbs block OSM, LIF and IL-6-dependent growth of plasmacytoma cells. Since OSM and LIF, as well as high levels of functional sCD126 [69] are found in vivo, it has been suggested that anti-CD130 may be therapeutically more effective than anti-CD126. In addition, it has been shown that an anti-CD126 MAb, AUK 64-7, which does not block the binding of IL-6 to its receptor, inhibits IL-6-dependent cell growth. Hence, this Ab may interfere with the interaction of CD126 and CD130 [43]. More recently, *Narazaki et al.* [70] have shown that natural sCD130 or recombinant sCD130 (which lacks a cytoplasmic domain) associates with the IL-6/sCD126 complex and inhibits the biological activities of IL-6, OSM, LIF and CNTF via interference with cytokine-signaling through membrane-anchored CD130.

Finally, various agents can modulate the production of IL-6 or the expression of CD126 [62]. The IL-1R antagonist, IL-4, corticosteroids, non-steroid anti-inflammatory agents and IL-6 antisense oligonucleotides have antitumor activity on myeloma cells by inhibiting IL-6 production [63, 71]. *Lamme et al.* [72] have demonstrated that some anti-adhesion molecule MAbs (anti-CD18, anti-CD29 and anti-CD44) block the adhesion of plasma cells to stromal cells and inhibit plasma cell-induced IL-6 production by BM stromal cells. IFN-gamma (IFN-γ) and all-trans retinoic acid also prevent tumor cell growth in vitro by downregulating the expression of CD126 on human myeloma cells [73, 74]. Suramin, a drug which interferes with the interaction of a number of different growth factors with their receptors, also prevents the binding of IL-6 to its receptor, and thus inhibits

the proliferative effects of IL-6 on human myeloma cell lines [75-77].

Tumor Cell Surface Molecules as Targets

Disease recurrence from autografted tumor cells is a major obstacle for ABMT in MM, even when the disease is in CR [78]. The potential use of anti-myeloma MAbs for ex vivo purging of these marrow grafts has been exploited using MAbs plus complement, MAbs bound to magnetic beads, or ITs [78-81]. These Abs or ITs may also be useful for treating patients [48].

Anderson et al. [79, 80] treated 40 MM patients with high-dose chemotherapy and either anti-B cell MAb-purged autologous or anti-T cell MAb-purged allogeneic BMT. The autologous BM was treated in vitro with anti-CD10, anti-CD20, anti-PCA-1 MAbs and rabbit complement to deplete tumor cells before transplantation. For allogeneic marrow grafts, T cells were depleted using anti-CD6 and complement. There were 18 (CRs), 18 partial responses and three toxic deaths. With 24 months of median follow-up, 16 of 26 patients were alive and stable at 2-55+ months. Of these, five patients remain in CR at 6-55+ months. In patients receiving allogeneic and syngeneic BMT, 8 of 14 patients are alive and stable at 8-34+ months. Although therapy has induced excellent responses and prolonged progression-free survival in some patients, relapses post-BMT suggest that treatment should be used earlier in the course of the disease.

Several ITs, containing MAbs recognizing plasma cell-associated antigens, and the ribosome-inactivating proteins momordin or saporin, have been used for ex vivo BM purging prior to ABMT [52, 53, 81]. One of these ITs (8A-momordin) also eliminates most of the S-phase B cells in the marrow which are postulated to be myeloma cell precursors. Fourteen patients with advanced disease have received 8A-momordin IT-purged autologous marrow. One is in clinical remission and three are in long-standing partial remission. Five died from myeloma recurrence or resistance, and another five patients died from infection or marrow failure [53].

It has been reported that peanut agglutinin lectin (PNA) selectively binds to plasma cells, but not to normal hematopoietic progenitors in the BM of 33 patients [82]. *Slupsky et al.* [83] have demonstrated that a major component of PNA-binding proteins on plasma cells is a novel, incompletely sialylated form of CD44. Further studies demonstrated that PNA-coated magnetic microspheres can selectively remove malignant plasma cells from BM without decreasing normal stem cell activity [84, 85]. By using a combination of PNA- and anti-CD19-coated magnetic beads for in vitro purging of autologous marrow, two patients with refractory advanced myeloma were treated with high-dose melphalan and total body-irradiation and then rescued with purged autologous marrow [86]. Both patients showed rapid engraftment of the purged marrow and remain well 36 and 46 months later with normal BM morphology, although one patient has a low level of circulating paraprotein. Ex vivo immunopurging of BM with avidin-biotin immunoabsorption or 4-hydroperoxycyclophosphamide (4-HC) has also been explored [87, 88]. Transplantation with purified autologous CD34-positive PBSC for MM is now being tested in the clinic [89].

Two antibody-enzyme conjugates, consisting of a free-radical-producing enzyme xanthine oxidase and MAb 62B1 or 8A recognizing an antigen expressed on differential B cells (hairy cells and plasma cells), have been reported to specifically kill human myeloma cells without damaging normal myeloid clonogenic efficiency [51, 78]. Since free radicals are produced on the surface of the target cells, these conjugates might be effective in killing tumor cells expressing antigens which are poorly internalized. This xanthine oxidase conjugate is not toxic in vivo because superoxide dismutase (SOD) and catalase in the blood prevent damage caused by free radicals which are generated by the enzyme [51, 78].

A humanized chimeric anti-CD38 Ab which consists of the Fab portion of the mouse MAb linked by a stable thioether bond to an Fc molecule derived from human IgG_1, has been prepared by *Stevenson et al.* [48]. The chimeric Ab mediates Ab-dependent cellular cytotoxicity (ADCC) very efficiently with human mononuclear effector cells either from normal donors or patients with florid myeloma and on various chemotherapeutic regimens. *Mathiot et al.* [90, 91] reported that sCD16, the low-affinity type III Fc receptor for IgG was significantly decreased in the sera of patients with MM and that this decrease correlated with the clinical stage of the disease. A recombinant sCD16 produced by *Teillaud et al.* [92] inhibited the proliferation and IgM/IgG production by human peripheral blood mononuclear cells stimulated

by pokeweed mitogen (PWM) in vitro. Recently, *Munshi et al.* [93] reported that recombinant sCD16 is lytic to human myeloma cells in vitro.

Guba et al. [47] have found that some myeloma cells coexpress B7 and CD28, and that anti-B7 Ab inhibits the growth of $CD28^+B7^+$ myeloma cells. These results suggest that the growth of myeloma cells may involve homotypic autostimulation via B7-CD28 interaction. In addition, anti-HLA-DR MAbs inhibit the proliferation of human myeloma cell lines by 50-100% [47]. *Hong et al.* [94] have reported that anti-major histocompatibility complex (MHC)-class II MAbs may induce transmembrane signals, and result in marrow graft failure in dogs, but the effect can be overcome by stem cell factor (SCF). Many studies have indicated that human myeloma cells express high levels of the intercellular adhesion molecule-1 (ICAM-1, CD54) [95]. We have recently demonstrated that an anti-CD54 MAb prevents the growth of a human myeloma cell line, ARH-77 in SCID mice although the mechanisms are not yet fully understood [96].

Drug resistance is a major obstacle for chemotherapy of MM. Overexpression of a transmembrane P-glycoprotein gp170 is the major mechanism for multi-drug resistance (MDR) in myeloma cells [97, 98]. *Tong et al.* reported that the chemosensitizing effect of cyclosporin A can be further enhanced by coadministration of anti-P-glycoprotein MAbs [99]. In addition, the anti-P-glycoprotein MAb, MRK16, plus rabbit complement effectively eliminated drug-resistant myeloma cells from human BM without affecting normal marrow stem cell survival [100, 101].

Immunomodulation

IFN-α has been used as induction therapy in previously untreated MM patients [57] and as maintenance therapy for patients with objective responses or stable disease [9, 10, 57]. It has also been combined with chemotherapy [9, 102, 103]. When used as a single agent, IFN-α has modest activity with an overall response rate of approximately 30% (range: 20-100%) and is less effective than conventional chemotherapy [57]. The effectiveness of combinations of IFN-α and chemotherapy is still controversial. The Myeloma Group of Central Sweden [9] has recently reported the results of a large randomized study comparing the efficacy of melphalan/prednisone (MP) therapy (n = 171) with MP plus natural IFN-α(MP/IFN, n = 164) in a series of 335 previously untreated patients. The response rate in the MP/IFN group was significantly higher than that in the MP group (68% versus 42%), but the overall survival rates were similar. However, in patients with IgA or Bence-Jones myeloma, a higher response rate in the MP/IFN group was observed (85% versus 48% for IgA myeloma and 71% versus 27% for Bence-Jones myeloma, respectively). The overall survival of the MP/IFN group was also significantly improved (median 32 versus 17 months). This study indicated that MP/IFN is superior to MP for inducing remissions and that it significantly improved the overall survival for patients with IgA and Bence-Jones myelomas. Similar results have also been reported by other investigators [13, 103, 104]. However, another randomized study in 278 patients failed to demonstrate an improvement in response or survival in patients undergoing MP/IFN treatment [102]. More promising results have been obtained by *Mandelli et al.* [10, 57] using IFN-α for maintenance therapy. They reported that maintenance treatment with IFN-α prolonged response and survival in patients who had responded to conventional induction therapy. There is only one clinical trial with IFN-γ in 15 MM patients, and no responses were observed in this study [105].

In vitro studies have indicated that both IFN-α and IFN-γ have effects on myeloma cells [74, 106-108]. It has been suggested that IFN-α may have direct cytostatic and/or cytotoxic effects on tumor cells [57]. Thus, at the termination of induction treatment, myeloma cells from responding patients are in G_0. IFN-α is capable of prolonging cell generation time, thereby reducing self-renewal of the cells [109]. IFN-α may also augment the immunogenicity of tumor cells by upregulating MHC class I antigens and tumor-associated antigens, and/or enhancing the host immune response by activating macrophages, T lymphocytes and NK cells [57]. Which of these activities is responsible for the clinical benefits from IFN-α remains uncertain. The antiproliferative activity of IFN-α is believed to be mediated through specific receptors, resulting in the induction of IFN response elements at cytoplasmic and nuclear levels. The exact mechanism is not firmly established but may involve a 2′5′ adenylate synthetase, protein kinase induction and downregulation of oncogene expression [57]. Several studies have indicated that IFN-α decreases both in vitro colony formation and the

LI of myeloma cells [106, 107] and can reduce their capacity for self-renewal [10], while IFN-γ inhibits the IL-6-dependent growth of fresh isolated myeloma cells and myeloma cell lines through downregulating the expression of IL-6Rs on tumor cells [74]. Indeed, IFN-γ inhibits only the proliferation of IL-6-dependent human myeloma cell lines. The sensitivity to IFN-α is not related to IL-6-dependent growth [74, 108, 110] and the combination of IFN-α plus IFN-γ and dexamethasone is the strongest inhibitor of myeloma growth and Ig secretion in vitro [110]. Therefore, IFN-γ in combination with IFN-α may have synergistic or additive effects.

IL-4 is a potent inhibitor of the expression of various inflammatory cytokines including IL-1, tumor necrosis factor (TNF), IL-8 and IL-6 [111-113]. *Taylor et al.* [114] have reported that IL-4 inhibits the clonogenic growth of myeloma cells in vitro. *Herrmann et al.* [115] have further demonstrated that IL-4 inhibits the growth of MM by suppressing IL-6 synthesis in BM cells from myeloma patients.

IL-2 has been used for many malignancies because it can induce the proliferation of T lymphocytes and NK cells, increase MHC-unrestricted cytotoxicity and induce the release of the cytokines TNF or IFN-γ which have antitumor effects [116]. The production of IL-2 from PBMC and the activity of NK cells in patients with aggressive or active myeloma are lower than in patients with indolent myeloma or in normal donors [117]. *Gottlieb et al.* [56] have demonstrated that malignant plasma cells isolated from patients with MM are sensitive to lysis by IL-2-induced LAK cells derived from the PBMCs of patients. The proliferation and survival of myeloma cells in vitro are reduced by TNF and IFN-γ released from the PBMCs in response to IL-2. Thus, in four patients treated with seven courses of IL-2 after ABMT, IL-2 infusion increased NK and LAK activity and induced production of TNF and IFN-γ from peripheral blood lymphocytes. However, cells capable of killing autologous myeloma cells did not circulate. *Pecherstorfer et al.* [118] reported that combined treatment of patients with chemotherapy-resistant MM with IL-2 and IFN-γ did not induce tumor regression but did increase the stimulation of NK cells. However, when low-doses of IL-2 were administered to 18 MM patients with advanced chemotherapy refractory disease, 6 of 17 patients experienced tumor regressions (2/17 objective responses, 4/17 long lasting stable disease following tumor progression before initiation of IL-2 treatment). Responders had a significantly lower plasma cell LI than nonresponders [119].

Activation of cytotoxic T cells by anti-CD3 is currently being evaluated in clinical trials as an approach to boosting anti-tumor effector mechanisms. *Massaia et al.* [120] have shown that the peripheral blood T cells from MM patients are very susceptible to stimulation with anti-CD3 MAbs. They have further demonstrated that anti-CD3-activated T cells from BM are able to generate potent anti-plasma cell activity in vitro. The anti-plasma cell activity of CD3-activated T cells involves cell-cell contact rather than cytokine-mediated effects [55]. A clinical trial in chemoreduced high-risk MM patients using OKT3 Ab plus IL-2 has shown that CD3 stimulation followed by IL-2 infusion induces significant T cell activation in vivo in MM patients [55]. An adoptive immunotherapeutic approach has also been explored using human NK cells [121]. The study showed that adoptive transfer of human NK cells with appropriate augmentation of activity by the cytokines IL-2 and IL-4 can inhibit tumor growth in SCID mice transplanted with the ARH-77 human myeloma cell line. These studies suggested that in vivo administration of cytokines such as IL-2 may have the potential for controlling minimal residual disease.

In addition, hematopoietic growth factors such as GM-CSF and granulocyte colony-stimulating factor (G-CSF) can ameliorate the BM suppressive effects of intensive chemotherapies and hasten stem cell engraftments and marrow reconstitution during marrow or PBSC transplantation [122, 123].

Conclusion

MM remains an incurable disease despite significant progress in understanding its biology and the development of new therapeutic approaches. New immunotherapies are promising, but they are still in preclinical studies or early clinical trials. Because agents with different mechanisms of action can exert synergistic effects on tumor cells with reduced side effects, future immunotherapy will probably consist of using various regimens involving MAbs and ITs against tumor-associated antigens and MAbs against

growth factors and their receptors. Cytokines and adoptive cellular therapy approaches will probably also be used in combination with each other and with chemotherapy. The importance of IL-6 and its receptor in the growth of myeloma cells and the accumulating evidence that MAbs against particular cell surface molecules can signal cell cycle arrest or apoptosis represent promising approaches. BM purging and the use of G-CSF or GM-CSF may eventually be performed as standard procedures in ABMT.

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Characterization of anti-mouse interleukin-6 receptor *antibody*. Okazaki Makoto; Yamada Yoshiki; Nishimoto Norihiro; Yoshizaki Kazuyuki; Mihara Masahiko
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10) Blockage of interleukin-6 receptor ameliorates joint disease in murine collagen-induced arthritis.
Takagi N; Mihara M; Moriya Y; Nishimoto N; Yoshizaki K; Kishimoto T; Takeda Y; Ohsugi Y
Chugai Pharmaceutical Company Ltd., Shizuoka, Japan.
Arthritis and rheumatism (UNITED STATES) Dec 1998, 41 (12) p2117-21, ISSN 0004-3591 Journal Code: 0370605
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
OBJECTIVE: To clarify the

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Clinical and experimental immunology (ENGLAND) Jun 1998, 112 (3) p397-402, ISSN 0009-9104 Journal Code: 0057202
Document type: Journal Article
Languages: ENGLISH

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Tamura T; Udagawa N; Takahashi N; Miyaura C; Tanaka S; Yamada Y; Koishihara Y; Ohsugi Y; Kumaki K; Taga T; et al
Department of Biochemistry, School of Dentistry, Showa University, Tokyo, Japan.
Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Dec 15 1993, 90 (24) p11924-8, ISSN 0027-8424 Journal Code: 7505876

13) Beneficial effects of pentoxifylline treatment of experimental acute *pancreatitis* in rats.
Marton J; Farkas G; Takacs T; Nagy Z; Szasz Z; Varga J; Jarmay K; Balogh A; Lonovics J
Department of Surgery, Albert Szent-Gyorgyi Medical University, Szeged, Hungary.
Research in experimental medicine. Zeitschrift fur die gesamte experimentelle Medizin einschliesslich experimenteller Chirurgie (GERMANY) 1998, 197 (5) p293-9, ISSN 0300-9130 Journal Code: 0324736 Document type: Journal Article

14) Experimental acute *pancreatitis* results in increased blood-brain barrier permeability in the rat: a potential role for tumor necrosis factor and interleukin 6.
Farkas G; Marton J; Nagy Z; Mandi Y; Takacs T; Deli M A; Abraham C S Department of Surgery, Albert Szent-Gyorgyi University Medical School, Szeged, Hungary. farkas@surg.szote.u-szeged.hu
Neuroscience letters (IRELAND) Feb 20 1998, 242 (3) p147-50, ISSN 0304-3940 Journal Code: 7600130
Document type: Journal Article
Languages: ENGLISH

15) Relevance of cytokine production to infected pancreatic necrosis. Farkas G; Nagy Z; Marton J; Mandi Y
Department of Surgery, Albert Szent-Gyorgyi Medical University, Szeged, Hungary.
Acta chirurgica Hungarica (HUNGARY) 1997, 36 (1-4) p86-8, ISSN 0231-4614 Journal Code: 8309977
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM

16) Peptide YY suppresses serum *IL*-*6* and TNF-alpha levels in murine necrotizing *pancreatitis*.
AUTHOR: Reed Kimberlee(a); McFadden David
AUTHOR ADDRESS: (a)Dep. Surg., UCLA Med. Cent., Los Angeles, CA**USA JOURNAL: Surgical Forum 48 (0):p179-180 1997
ISSN: 0071-8041
RECORD TYPE: Citation
LANGUAGE: English
1997

Thanks.

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ART UNIT 1647 09/762550

Acta Chirurgica Hungarica, 36 (1-4) pp. 86-88 (1997)

RELEVANCE OF CYTOKINE PRODUCTION TO INFECTED PANCREATIC NECROSIS

G. Farkas, Zs. Nagy*, J. Márton and Yvett Mándi*

*Department of Surgery and *Institute of Microbiology, Albert Szent-Györgyi Medical University H-6701 Szeged, P.O. Box 464, Hungary*

The purpose of this study was to evaluate the role of cytokines in septic conditions following acute pancreatitis and to elaborate a new strategy in the treatment. Increased TNF and IL-6 serum levels were found in 30% of the patients (n=40), while the IL-6 level was elevated in all of them. There was a positive correlation between the serum IL-6 and sICAM-1 levels. The *in vitro* TNF and IL-6 producing capacities were initially higher in the study group, but decreased on subsequent days, especially in fatal cases (n=3). Administration of pentoxifylline [PTX] (400 mg/day) to septic patients following necrotizing pancreatitis resulted in TNF and IL-6 production similar to that observed in control donors. The level of sICAM-1 also decreased following PTX therapy. These results suggest that cytokines produced by activated leucocytes are important in the pathogenesis of infected pancreatic necrosis, and their inhibition might be of therapeutic advantage.

Introduction

Sepsis and septic shock are the most frequent complications of extended necrosis following acute pancreatitis. The purpose of this study was to evaluated the role of cytokines in these conditions and to elaborate a new strategy in the treatment. Tumor necrosis factor (TNF) plays a pivotal role in the initiation of septic syndrome [1]. TNF is produced mainly by monocytes, macrophages and granulocytes in response to various stimuli, of which endotoxins derived from Gram negative bacteria, are the most potent. Serum TNF, IL-1ß, IL-6, ICAM levels and the TNF-producing capacity of the leucocytes has been checked in patients with presumed sepsis following necrotizing pancreatitis. Recently, hemorrheologic agent pentoxifylline [PTX] has been shown to inhibit TNF production [2]. We therefore investigated the effects of PTX not only on TNF and IL-6 synthesis *in vitro* and in septic patients in clinical practice but also on the serum level of soluble ICAM-1 (sICAM-1) [3].

Patients and Methods

Forty patients with infected pancreatic necrosis were studied. All patients had positive cultures from the abdominal fluid and abscess discharge with Gram negative rods. 25 of the forty patients had positive blood cultures as well. In all patients the surgical

0231-4614 /97/ $ 5.00

treatment consisted of wide-ranging necrosectomy combined with continuous widespread washing and suction drainage. PTX has been administered in an open unblinded study in a dose of 400 mg/day as a complement to conventional antibiotic therapy. The results were compared with those of earlier conventional intensive therapy without PTX (n=20). The severity of the illness was evaluated in accordance with the APACHE II score system. *Titration of TNF and IL-1ß* in the sera was measure by TNF ELISA and IL-1ß ELISA (Medgenix®) respectively. TNF content in cell supernatants was checked in bio-assay based on cell line WEHI 164 clone 13. TNF activities were calibrated against recombinant human TNF (Genentech®). *IL-6 assay* was performed using the IL-6 dependent B-9 cell line proliferation test. *Determination of sICAM-1* was performed by sICAM-1 ELISA (Bender Med. System, Vienna), according to the instruction of the manufacturer. *Stimulation of leucocytes:* whole blood was incubated for 24 hr at 37 °C with heat killed Staphylococcus aureus or with E. coli LPS (Sigma®) respectively. The supernatants were tested for the presence of TNF.

Results

Initial serum samples for TNF, IL-1ß and IL-6 determination were obtained between 12 and 20 hr after onset of sepsis, following by daily sampling. Of the serum samples taken, only 30% of patients contained detectable TNF. All serum samples positive for TNF were obtained within 48 hr of onset of the clinical signs of sepsis. The similar tendency in the kinetic of appearance of IL-1ß in the serum could be observed (50% of the patients vs. 30% of patients on day 1 and day 2). In contrast to IL-1ß and TNF, IL-6 serum levels were almost always above normal value in septic patients. The mean peak value of TNF in septic patients was 110 pg/ml and there was no significant difference between survivors and nonsurvivors. The peak value of IL-1ß did not differ between survivors and nonsurvivors as well. The serum levels of IL-6 seemed to be correlated with the severity of illness (250 U/ml vs. 400 U/ml peak value). The level of sICAM-1 was the highest among nonsurvivors in average 2000 ng/ml. There was a positive correlation between serum IL-6 and sICAM-1 levels ($r=0.6837$, $p < 0.001$). Stimulation of whole blood cultures with Staphylococcus aureus led to higher TNF production than it was measured in the control group. The follow-up study of septic patients revealed that in vitro TNF production decreased in later phase of disease, on correlation of severity of illness. PTX significantly decreased the TNF production of mononuclear cells in a dose-dependent manner when either LPS or Staphylococcus aureus was the inducer. In contrast, there was only a moderate decrease in IL-6 production. Beneficial effect of PTX in septic syndrome was observed following necrotizing pancreatitis. As a consequence of PTX therapy, the TNF production dropped to the normal level on day 2. There was a moderate decrease in the level of IL-6 production. The circulating sICAM-1 level was significantly higher in septic patients then in normal controls and it remained elevated for the subsequent days. PTX administration resulted in a rapid decrease in sICAM-1 level. The severity of illness was evaluated in accordance with the APACHE II

score system, PTX therapy resulted in a decreasing tendency in the scores, which tended to change inversely with the improvement in the clinical status, and the laboratory parameters.

Discussion

Using a bio-assay or ELISA a revealed circulating TNF in 30% of patients with infected pancreatic necrosis. There was no clear association of TNF level and the development of septic shock or fatal outcome of the disease. The *in vitro* TNF-producing capacity was high in the patients in the study group. The decrease in inducibility before the fatal outcome of the disease might be due to the exhaustion of the leukocytes. This decrease of responsiveness might be of prognostic value. Our results suggest that determination of the TNF producing capacity of the leucocytes might be more informative than measurement of the serum TNF level in evaluation of the severity or prognosis of sepsis. In our studies, the production of TNF was inhibited by PTX not only after LPS stimuli, but also after stimulation of effector cells by heat-killed Staphylococcus aureus. The circulating or soluble intercellular adhesion molecule (sICAM-1) reflects a state of general cell activation, or can be regarded as a marker of the presence of inflammatory madiators and cytokines. A decrease in sICAM-1 was observed in parallel with decrease in APACHE II score of our patients treated with PTX. PTX may therefore improve therapeutic strategies in septic complications following necrotizing pancreatitis.

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? b 155
05aug03 09:31:15 User217743 Session D614.2
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$0.00 Estimated cost File410
$0.00 Estimated cost this search
$0.01 Estimated total session cost 0.232 DialUnits
File 155:MEDLINE(R) 1966-2003/Aug W1
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Set Items Description
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63 IL?6
427462 RECEPTOR
0 IL?6(W)RECEPTOR
3 ANTAGONIS
S1 0 IL?6()RECEPTOR AND ANTAGONIS
? s IL?6()receptor and antagonist
63 IL?6
427462 RECEPTOR
0 IL?6(W)RECEPTOR
105405 ANTAGONIST
S2 0 IL?6()RECEPTOR AND ANTAGONIST
? s (IL6 or Il()6)()receptor
1036 IL6
109583 IL
1474437 6
21845 IL(W)6
427462 RECEPTOR
S3 1338 (IL6 OR IL()6)()RECEPTOR
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1338 S3
105405 ANTAGONIST
S4 78 S3 AND ANTAGONIST
? s s4 and (antibody or antibodies)
78 S4
403228 ANTIBODY
422038 ANTIBODIES
S5 21 S4 AND (ANTIBODY OR ANTIBODIES)
? s s5 and py>1999
21 S5
1822327 PY>1999
S6 8 S5 AND PY>1999
? s s5 not s6
21 S5
8 S6
S7 13 S5 NOT S6

? t s7/3,ab/all

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11879317 99321011 PMID: 10395088
A monoclonal *antibody* recognizing an epitope shared by receptors for growth hormone, prolactin, interleukin 2 and interleukin 6. Longhi S A; Miranda M E; Gobet M G; Retegui L A
Instituto de Quimica y Fisicoquimica Biologicas (UBA-CONICET), Facultad de Farmacia y Bioquimica, Buenos Aires, Argentina.
Molecular and cellular biochemistry (NETHERLANDS) May 1999, 195 (1-2) p235-43, ISSN 0300-8177
Journal Code: 0364456
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Monoclonal *antibody* (MAb) termed R7B4 was generated throughout the idiotypic-anti-idiotypic network from mice immunized with human and bovine growth hormones (GH). The Ab was selected on the basis that it did not recognize human GH (hGH) neither insolubilized nor in solution but inhibited 125I-hGH binding to receptors from rat and rabbit liver and from Nb2-cell membranes. Since it inhibited Nb2-cell mitogenesis stimulated by hGH, prolactins or placental lactogens, MAb R7B4 behaved as an *antagonist* of lactogenic hormones. Furthermore, the Ab impaired proliferative activity of interleukin 2 (IL-2) on Nb2 cells as well as growth of 7TD1 cells, an interleukin 6 (IL-6) dependent hybridoma not expressing GH receptors. Biotin-labeled MAb R7B4 specifically bound to rat liver microsomes, and the Ab was able to recognize Nb2 and 7TD1-cell membranes as shown by flow cytometry experiments. However, MAb binding was not hampered by hGH, indicating that the Ab did not mimic GH binding site to receptors. Immunoblot assays indicated that rat and rabbit liver as well as Nb2-cells membrane antigens recognized by MAb R7B4 were similar to those revealed by a MAb directed to prolactin receptors. In addition, MAb R7B4 was able to detect two bands probably corresponding to the somatogenic receptor in rabbit liver microsomes as well as three different proteins in 7TD1-cells showing molecular weights similar to those of the *IL*- *6* *receptor* complex. Results suggest that MAb R7B4 is directed to an epitope shared by receptors for lactogenic and somatogenic hormones, IL-2 and IL-6. To our knowledge, these data are the first experimental evidence of the existence of structural similarity between some of the receptors grouped in the cytokine receptor superfamily.

7/3,AB/2
DIALOG(R)File 155:MEDLINE(R)
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11789909 99228628 PMID: 10210772
Interleukin-6 receptor signaling. I. gp80 and gp130 receptor interaction in the absence of interleukin-6.
Gaillard J P; Mani J C; Liautard J; Klein B; Brochier J
INSERM U. 475, 99, rue Puech-Villa, 34197 Montpellier, Cedex 05 France. European cytokine network (FRANCE) Mar 1999, 10 (1) p43-8, ISSN 1148-5493 Journal Code: 9100879
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Interleukin-6 (IL-6) is used as a growth factor by various tumor cells. It binds to a gp80 specific receptor (IL-6R) and then to a gp130 transducing chain. Both receptor chains are released as soluble functional proteins which circulate in biological fluids. To study the physiological role of these soluble receptors, both proteins were purified from human plasma and the kinetic constants of equilibria between IL-6 and its natural soluble IL-6R (sIL-6R) and gp130 receptor (sgp130) were measured using surface plasmon resonance analysis. Unexpectedly, natural sIL-6R and natural sgp130 were found to interact (Kd = 2.8 nM) in the absence of IL-6. No interaction was seen between the recombinant soluble receptors or between either natural soluble receptor and its recombinant partner. This binary complex was not due to copurification of IL-6 and was detected in human plasma of healthy donors. It results from either direct interaction between the two natural soluble receptors or indirect binding mediated by a yet unidentified copurified plasma molecule playing the role of an IL-6 *antagonist*. Once formed, the binary complex was found to be unable to bind IL-6. Soluble gp130 had already been shown to inhibit IL-6 signaling by inactivating the IL-6/IL-6R complex. In addition we show that, in the absence of IL-6, circulating natural sgp130 is able to inhibit directly the circulating sIL-6R that is a strong synergic molecule of IL-6 signaling.

7/3,AB/3
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11683466 99118958 PMID: 9921985
Blocking signaling through the Gp130 receptor chain by interleukin-6 and oncostatin M inhibits PC-3 cell growth and sensitizes the tumor cells to etoposide and cisplatin-mediated cytotoxicity.
Borsellino N; Bonavida B; Ciliberto G; Toniatti C; Travali S; D'Alessandro N
Istituto di Farmacologia, Policlinico P. Giaccone, Universita di Palermo, Italy.
Cancer (UNITED STATES) Jan 1 1999, 85 (1) p134-44, ISSN 0008-543X Journal Code: 0374236
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
BACKGROUND: The mechanisms of drug resistance associated with advanced, hormone-independent prostate carcinoma are poorly understood. The human prostate carcinoma PC-3 cell line, derived from a metastatic tumor and lacking androgen receptors, represents a useful model to investigate drug resistance. METHODS: The effects of oncostatin M (OM), antiinterleukin-6 (IL-6) treatment, or interference with the gp130-mediated signaling on etoposide- or cisplatin-mediated cytotoxicity were investigated. RESULTS: Both endogenous and exogenous IL-6 and exogenous OM up-regulated cell growth and enhanced resistance of PC-3 tumor cells to both etoposide and cisplatin. The influence of IL-6 is controlled by treating PC-3 tumor cells with anti-IL-6 neutralizing *antibody* and, more efficiently, by a mutated IL-6, Sant7. Sant7 has a high affinity binding to the *IL*- *6* *receptor*-alpha (IL-6Ralpha) subunit, but does not bind to the signaling subunit gp130; therefore, it behaves as a receptor *antagonist* . Both IL-6- and OM-mediated effects are inhibited by the treatment of PC-3 with an antisense oligodeoxynucleotide against gp130, the protein kinase inhibitor genistein (GNS), or the monoterpene perillic acid (PA), a posttranslational inhibitor of p21ras isoprenylation. CONCLUSIONS: These results demonstrate the protective roles in drug sensitivity of IL-6 and OM through signaling of the common chain gp130 and, most likely, a downstream ras-dependent pathway in PC-3 tumor cells. These findings suggest the potential clinical application of anticytokine therapy or interference with gp130 signaling in the treatment of drug resistant prostate carcinoma.

7/3,AB/4
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11276408 98154898 PMID: 9495510
LIF, but not IL-6, regulates osteoprogenitor differentiation in rat calvaria cell cultures: modulation by dexamethasone.
Malaval L; Gupta A K; Liu F; Delmas P D; Aubin J E
INSERM U403, Hopital Edouard Herriot, Lyon, France.
Journal of bone and mineral research - the official journal of the American Society for Bone and Mineral Research (UNITED STATES) Feb 1998, 13 (2)

p175-84, ISSN 0884-0431 Journal Code: 8610640
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Cytokines of the interleukin 6 (IL-6) subfamily are a group of factors produced by osteoblasts and acting through the same transducing element, membrane protein gp130. We have previously shown that exogenous (added to the culture medium) leukemia inhibitory factor (LIF) inhibits bone nodule formation and expression of osteoblast-associated genes in fetal rat calvaria (RC) cell cultures and that dexamethasone (Dex) increases the ID50 of LIF. To investigate the respective roles of IL-6-related cytokines and receptors in osteprogenitor differentiation, and their regulatory interplay with Dex, we used reverse transcribed polymerase chain reaction, bioassay, and blocking *antibody* techniques to assess the time courses of LIF, IL-6, LIF transmembrane receptor, *IL*-*6* *receptor*, and gp130 expression in RC cell cultures grown with and without Dex. The levels of the mRNAs for IL-6, LIF, and gp130 decreased concomitantly with the formation of bone nodules. Dex treatment, which stimulates bone nodule formation, reduced the expression of LIF and IL-6 mRNAs and IL-6 bioactivity in the culture medium. LIF treatment strongly stimulated the expression of IL-6. Incubation with anti-LIF *antibodies* increased the number of nodules, while an *antibody* blocking IL-6 activity had little or no effect on nodule numbers and did not antagonize the action of exogenous LIF, indicating that IL-6 does not mediate the action of LIF in this system. Moreover, although exogenously added IL-6 was active in the cultures as noted by a reduction of nodule mineralization, it had no effect on nodule numbers, i.e., on osteoprogenitor differentiation, in the presence or absence of Dex. In conclusion, IL-6, LIF, and their receptors are expressed throughout the time-course of osteogenesis in RC cell cultures. However, only LIF, but not IL-6, appears to play a significant role in autocrine regulation of osteoblastic differentiation in this system. The *antagonist* action of Dex on the effects of exogenously added LIF, as well as the bone-promoting action of Dex in RC cell cultures, could be exerted partly through the down-regulation of the expression of endogenous LIF.

7/3,AB/5
DIALOG(R)File 155:MEDLINE(R)

10803491 97093003 PMID: 8938565
Immunomodulatory and hematopoietic effects of recombinant human interleukin-6 in patients with advanced renal cell cancer. Schuler M; Peschel C; Schneller F; Fichtner J; Farber L; Huber C; Aulitzky W E
Department of Medicine III, Johannes Gutenberg University, Mainz, Germany.
Journal of interferon & cytokine research - the official journal of the International Society for Interferon and Cytokine Research (UNITED STATES) Nov 1996, 16 (11) p903-10, ISSN 1079-9907 Journal Code: 9507088
Document type: Clinical Trial; Clinical Trial, Phase II; Journal Article; Multicenter Study
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Interleukin-6 (IL-6) is a cytokine with pleiotropic biologic activities on B cells, T cells, and hematopoietic progenitors. The present study was undertaken to assess pharmacodynamic effects of subcutaneous administration of IL-6 on blood counts, immunologic parameters, and acute-phase reactants. Blood samples were taken from patients with advanced renal cell cancer participating in a phase II trial of recombinant human IL-6. Multiparameter FACS analyses of peripheral blood mononuclear cells were performed using *antibodies* against CD3, CD4, CD8, HLA-DR, CD56, CD28, CD38, CD19, sIgM, and sIgG. Serum levels of IL-10, soluble CD23 (sCD23), sCD25, IL-1 receptor *antagonist* protein (IL-1RA), soluble tumor necrosis factor (TNF) receptors (sTNF-R) p55 and p75, and soluble *IL*-*6* *receptor* (sIL-6R) were detected by ELISA systems. Levels of C-reactive protein (CRP), neopterin, fibrinogen, beta 2-microglobulin, and immunoglobulins M, G, and A were measured by standard methods. In response to administration of IL-6, a significant increment in platelet counts was observed, reaching peak levels after 21 days of treatment. In contrast, leukocyte subsets remained unaffected. No change in number of immunophenotype of peripheral blood B cells, T cells, or natural killer cells could be detected following IL-6 administration. Blood levels of sCD23, IL-10, sIL-6R, neopterin, beta 2-microglobulin, and immunoglobulin subsets were not influenced by cytokine therapy. However, administration of IL-6 led to a slow increment of acute-phase reactants CRP and fibrinogen. Furthermore, the anti-inflammatory molecules sTNF-R p55 and p75 were induced by IL-6, whereas serum levels of IL-1RA remained unchanged. Finally, an increase in blood levels of sCD25 was observed. In conclusion, IL-6 in vivo predominantly acts as a regulator of inflammation and a megakaryocyte differentiation factor.

7/3,AB/6
DIALOG(R)File 155:MEDLINE(R)

10743491 97093003 PMID: 8938565
Immunomodulatory and hematopoietic effects of recombinant human interleukin-6 in patients with advanced renal cell cancer. Schuler M; Peschel C; Schneller F; Fichtner J; Farber L; Huber C; Aulitzky W E
Department of Medicine III, Johannes Gutenberg University, Mainz, Germany.
Journal of interferon & cytokine research - the official journal of the International Society for Interferon and Cytokine Research (UNITED STATES) Nov 1996, 16 (11) p903-10, ISSN 1079-9907 Journal Code: 9507088
Document type: Clinical Trial; Clinical Trial, Phase II; Journal Article; Multicenter Study
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Interleukin-6 (IL-6) is a cytokine with pleiotropic biologic activities on B cells, T cells, and hematopoietic progenitors. The present study was undertaken to assess pharmacodynamic effects of subcutaneous administration of IL-6 on blood counts, immunologic parameters, and acute-phase reactants. Blood samples were taken from patients with advanced renal cell cancer participating in a phase II trial of recombinant human IL-6. Multiparameter FACS analyses of peripheral blood mononuclear cells were performed using *antibodies* against CD3, CD4, CD8, HLA-DR, CD56, CD28, CD38, CD19, sIgM, and sIgG. Serum levels of IL-10, soluble CD23 (sCD23), sCD25, IL-1 receptor *antagonist* protein (IL-1RA), soluble tumor necrosis factor (TNF) receptors (sTNF-R) p55 and p75, and soluble *IL*-*6* *receptor* (sIL-6R) were detected by ELISA systems. Levels of C-reactive protein (CRP), neopterin, fibrinogen, beta 2-microglobulin, and immunoglobulins M, G, and A were measured by standard methods. In response to administration of IL-6, a significant increment in platelet counts was observed, reaching peak levels after 21 days of treatment. In contrast, leukocyte subsets remained unaffected. No change in number of immunophenotype of peripheral blood B cells, T cells, or natural killer cells could be detected following IL-6 administration. Blood levels of sCD23, IL-10, sIL-6R, neopterin, beta 2-microglobulin, and immunoglobulin subsets were not influenced by cytokine therapy. However, administration of IL-6 led to a slow increment of acute-phase reactants CRP and fibrinogen. Furthermore, the anti-inflammatory molecules sTNF-R p55 and p75 were induced by IL-6, whereas serum levels of IL-1RA remained unchanged. Finally, an increase in blood levels of sCD25 was observed. In conclusion, IL-6 in vivo predominantly acts as a regulator of inflammation and a megakaryocyte differentiation factor.

7/3,AB/7
DIALOG(R)File 155:MEDLINE(R)

10532448 96343846 PMID: 8760789
Histamine selectively enhances human immunoglobulin E (IgE) and IgG4 production induced by anti-CD58 monoclonal *antibody*. Kimata H; Fujimoto M; Ishioka C; Yoshida A
Department of Pediatrics, Unitika Central Hospital, Kyoto, Japan. Journal of experimental medicine (UNITED STATES) Aug 1 1996, 184 (2) p357-64, ISSN 0022-1007 Journal Code: 2985109R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We studied the effects of histamine on human immunoglobulin (IgE) and IgG4 production. Histamine selectively enhanced IgE and IgG4 production in purified surface IgE and IgG4 negative (sIgE-sIgG4-) B cells from normal donors stimulated with interleukin (IL)-4 plus anti-CD58 or IL-13 plus anti-CD58 monoclonal *antibody* (mAb) without affecting production of IgG1, IgG2, IgG3, IgM, IgA1, or IgA2. In cultures with IL-4 plus anti-CD58 mAb, histamine-induced enhancement of IgE and IgG4 production was specifically blocked by thioperamide (H3 receptor *antagonist*), and was inhibited by anti-IL-10 *antibody* (Ab). In contrast, in cultures with IL-13 plus anti-CD58 mAb, histamine-induced enhancement was blocked by dimaprit (H1 receptor *antagonist*), and was inhibited by anti-IL-6 mAb. Histamine also enhanced IgE and IgG4 production by in vivo-generated sIgE+ and sIgG4+ B cells, respectively, from atopic patients; enhancement was blocked by dimaprit and thioperamide, and was inhibited by anti-IL-6 mAb and anti-IL-10 Ab. In sIgE-sIgG4- B cells, IL-4 plus anti-CD58 mAb induced IL-10 production and IL-10 receptor expression, whereas IL-13 plus anti-CD58 mAb induced IL-6 production and *IL*-*6* *receptor* expression. Histamine increased IL-10 and IL-6 production without affecting IL-10 and *IL*-*6* *receptor* expression, in cultures with IL-4 plus anti-CD58 mAb and with IL-13 plus anti-CD58 mAb, respectively, which was blocked by thioperamide and dimaprit, respectively. In contrast, sIgE+ and sIgG4+ B cells spontaneously produced both IL-6 and IL-10 and constitutively expressed IL-6 and IL-10 receptors, and histamine increased IL-6 and IL-10 production without affecting IL-6 or IL-10 receptor expression, which was blocked by thioperamide and dimaprit. These results indicate that histamine enhanced IgE and IgG4 production by increasing endogenous IL-6 and IL-10 production via H1 and H3 receptors, respectively.

7/3,AB/8

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10408775 96215000 PMID: 8621403
The interleukin-6 (IL-6) partial *antagonist* (Q159E,T162P)IL-6 interacts with the *IL*-*6* *receptor* and gp130 but fails to induce a stable hexameric receptor complex.
Hammacher A; Simpson R J; Nice E C
Joint Protein Structure Laboratory, Ludwig Institute for Cancer Research and Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria 3050, Australia.
Journal of biological chemistry (UNITED STATES) Mar 8 1996, 271 (10) p5464-73, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The extracellular "soluble" domains of the *IL*-*6* *receptor* (sIL-6R) and gp130 (sgp130) form a hexameric ternary receptor complex together with IL-6, consisting of two molecules of each component. In this report we have investigated the interactions of the partial IL-6 *antagonist* (Q159E,T162P)IL-6 ((QT)IL-6), with the sIL-6R and sgp130. The kinetic rate constants of the binding of sIL-6R to immobilized monomeric (QT)IL-6 or IL-6 were obtained using an optical biosensor with analysis of the primary data by linear and nonlinear regression. Both methods of analysis showed that, due to a higher off-rate, sIL-6R has lower apparent affinity for (QT)IL-6 than IL-6. The lower affinity of (QT)IL-6 was further confirmed by equilibrium binding measurements at the sensor surface and in solution. Using the biosensor it was also shown that the (QT)IL-6 complex interacts with sgp130, supporting the notion that the biological activity of (QT)IL-6 is mediated via gp130. However, the IL-6 mutant, when incubated with sIL-6R and sgp130, failed to induce a stable hexameric receptor complex, as shown by narrowbore size exclusion chromatography.

7/3,AB/9
DIALOG(R)File 155:MEDLINE(R)
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10356709 96159494 PMID: 8589270
Epitope peptides from interleukin-6 receptor which inhibit the growth of human myeloma cells.
Halimi H; Eisenstein M; Oh J W; Revel M; Chebath J
Department of Molecular Genetics and Virology, Weizmann Institute of Science, Rehovot, Israel.
European cytokine network (FRANCE) May-Jun 1995, 6 (3) p135-43, ISSN 1148-5493 Journal Code: 9100879
Erratum in Eur Cytokine Netw 1995 Jul-Dec;6(4) 219
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
A panel of monoclonal *antibodies* against the soluble *IL*- *6* *receptor* was used to search for linear epitopes by a Pepscan analysis. Two such epitopes were found and the corresponding peptides were synthesized chemically. The peptides were active to inhibit the IL-6 dependent growth of human multiple myeloma cell line and the effect of IL-6 on growth of murine hybridoma cells. The epitope-defined, *antagonist* peptides reduced the transduction of the IL-6 signal which activates binding of Stat transcription factors to specific enhancers, but did not affect IL-6 binding. These effects were not seen with several other peptides from the *IL*-*6* *receptor* sequence. A computer three-dimensional model of the *IL*-*6* *receptor* complex was built and indicates that the *antagonist* peptides define one of the two possible sites of interaction between the domain-II of the *IL*-*6* *receptor* molecule and that of the gp130 molecule within the hexameric receptor assembly.

7/3,AB/10
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.

10288184 96090049 PMID: 7579076
Angiotensin II stimulates interleukin-6 release from cultured mouse mesangial cells.
Moriyama T; Fujibayashi M; Fujiwara Y; Kaneko T; Xia C; Imai E; Kamada T; Ando A; Ueda N
First Department of Medicine, Osaka University School of Medicine, Japan. Journal of the American Society of Nephrology - JASN (UNITED STATES) Jul 1995, 6 (1) p95-101, ISSN 1046-6673 Journal Code: 9013836
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Interleukin-6 (IL-6) is a multifunctional cytokine exerting a wide variety of biologic responses, including cell proliferation. Recently, IL-6 has been known to play a role in the pathogenesis of mesangial proliferative glomerulonephritis. IL-6 is now recognized as an autocrine growth factor for glomerular mesangial cells, and various inflammatory mediators have been shown to promote IL-6 release from mesangial cells. However, little is known about the noninflammatory stimuli of IL-6 release from mesangial cells. In this study, it was hypothesized that angiotensin II (AngII) is one of the noninflammatory mediators of IL-6 release in mesangial cells, and the effects of AngII on IL-6

release and mRNA expression in cultured mouse mesangial cells (CMMC) were investigated. It was demonstrated that AngII (10(-7) M or higher) caused IL-6 release and mRNA accumulation in CMMC. IL-6 release was detected at 4 h and reached a plateau at 8 h after the addition of AngII, whereas IL-6 mRNA expression peaked at 4 h. The effects of AngII on IL-6 release and gene expression were completely blocked by the AngII receptor type 1 (AT1 receptor) *antagonist* CV-11974. AngII and IL-6 were both shown to stimulate DNA synthesis in CMMC, and the blockade of IL-6 signaling with anti-*IL*- *6* *receptor* *antibody* abolished the enhanced DNA synthesis induced by AngII. These results raise a possibility that the growth-promoting effect of AngII on mesangial cells is at least partially mediated by IL-6 released from mesangial cells.

7/3,AB/11
DIALOG(R)File 155:MEDLINE(R)

10274008 96075789 PMID: 7578977
Functional distinction of two regions of human interleukin 6 important for signal transduction via gp130.
de Hon F D; ten Boekel E; Herrman J; Clement C; Ehlers M; Taga T; Yasukawa K; Ohsugi Y; Kishimoto T; Rose-John S; et al
Department of Autoimmune Diseases, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam.
Cytokine (UNITED STATES) Jul 1995, 7 (5) p398-407, ISSN 1043-4666 Journal Code: 9005353
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Mutagenesis of a region of human interleukin (IL)-6 which is important for triggering signal transduction via the *IL*-*6* *receptor* beta-chain (gp130) has lead to the isolation of a variant of human IL-6 (IL-6.Q160E/T163P), which could antagonize the biological activity of wild type IL-6 on the human EBV transformed B cell line CESS and the human hepatoma cell line HepG2. Surprisingly this antagonistic IL-6 variant had an agonistic effect on the human myeloma cell line XG-1, albeit at a 1000-fold higher concentration than wild type IL-6. This residual activity of the mutant arose from triggering gp130, because it could be inhibited by a gp130 specific mAb. Extensive mutagenesis of residues between Q153 and H165 of human IL-6, a region which is partly homologous in cytokines which also signal via gp130 (oncostatin M, ciliary neurotrophic factor, leukaemia inhibitory factor, IL-11), did result in the isolation of a second *antagonist* for IL-6 activity on CESS and HepG2 cells. However on XG-1 cells this variant was active as well. These results suggest that (an) additional region(s) of the IL-6 molecule might be involved in gp130 triggering. Recently we indeed found that residues Lys42-Ala57 are also important for gp130 triggering. Inhibition experiments with neutralizing IL-6R alpha-chain specific mAb show that this region can be functionally separated from the Q153-H165 region. These findings have important implications for the development of receptor antagonists of IL-6 and IL-6 family members.

7/3,AB/12
DIALOG(R)File 155:MEDLINE(R)

08618957 95307484 PMID: 7540468
Immunotherapy of multiple myeloma.
Huang Y W; Vitetta E S
Cancer Immunobiology Center, University of Texas Southwestern Medical Center, Dallas.
Stem cells (Dayton, Ohio) (UNITED STATES) Mar 1995, 13 (2) p123-34, ISSN 1066-5099 Journal Code: 9304532
Document type: Journal Article; Review; Review, Academic Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
In 1994, an estimated 12,700 new cases of multiple myeloma (MM) will be diagnosed in the USA and 9,800 patients will die from this disease. At present, a cure for MM has not been achieved with any chemotherapeutic regimen. Therefore, it is important to develop novel therapeutic approaches to treat this fatal disease. This review focuses on new concepts in the immunotherapy of MM. Thus far, interferons and anti-human interleukin (IL)-6 monoclonal anti-bodies (MAbs) have been used to treat patients with this disease. Bone marrow transplantation using autologous marrow purged with MAbs and complement, with anti-myeloma immunotoxins (ITs), or MAb-magnetic bead conjugates has been reported. Adoptive cellular therapy, in vivo with anti-CD3 and IL-2, as well as transplantation of purified autologous CD34+ peripheral blood stem cells, is now being evaluated in clinical trials. Anti-human *IL*-*6* *receptor* (IL-6R) and anti-CD54 (ICAM-1) MAbs have shown promising results in the therapy of human myeloma cell lines in SCID mice, while an IL-6 *antagonist* protein, anti-gp130 MAbs, recombinant soluble gp130, anti-B7, anti-HLA-DR, and recombinant soluble CD16 also inhibit the growth of myeloma cell lines in vitro. These experimental therapeutic modalities hold promise for use in humans and may also provide further insights into the pathogenesis of MM.

7/3,AB/13
DIALOG(R)File 155:MEDLINE(R)

08037579 94103309 PMID: 8276883
Development of a human interleukin-6 receptor *antagonist*. Brakenhoff J P; de Hon F D; Fontaine V; ten Boekel E; Schooltink H; Rose-John S; Heinrich P C; Content J; Aarden L A
Department of Autoimmune Diseases, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam.
Journal of biological chemistry (UNITED STATES) Jan 7 1994, 269 (1) p86-93, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Neutralizing monoclonal *antibodies* specific for human interleukin-6 (IL-6) bind two distinct sites on the IL-6 protein (sites I and II). Their interference with *IL*-*6* *receptor* binding suggested that site I is a receptor-binding site of IL-6, whereas site II is important for signal transduction. Mutagenesis of site II could therefore result in the isolation of *IL*-*6* *receptor* antagonists. To test this hypothesis, a panel of IL-6 mutant proteins was constructed that did not bind to a site II-specific monoclonal *antibody*. One such site II mutant protein (with double substitution of Gln-160 with Glu and Thr-163 with Pro) was found to be an *antagonist* of human IL-6. It was inactive on human CESS cells, weakly active on human HepG2 cells, but active on mouse B9 cells. It could specifically antagonize the activity of wild-type IL-6 on CESS and HepG2 cells. The binding affinity of this variant for the 80-kDa *IL*-*6* *receptor* was similar to that of wild-type IL-6. High affinity binding to CESS cells, however, was abolished, suggesting that the mutant protein is inactive because the complex of the 80-kDa *IL*-*6* *receptor* and the mutant protein cannot associate with the signal transducer gp130. The human IL-6 *antagonist* protein may be potentially useful as a therapeutic agent. ? logoff y
05aug03 09:36:27 User217743 Session D614.3
$3.64 1.137 DialUnits File155
$2.73 13 Type(s) in Format 4 (UDF)
$2.73 13 Types
$6.37 Estimated cost File155
$1.40 TELNET
$7.77 Estimated cost this search
$7.78 Estimated total session cost 1.369 DialUnits
Logoff: level 02.18.00 D 09:36:27

$%^Dialog;HighlightOn=*;HighlightOff=*;

Connecting via Winsock to Dialog

Logging in to Dialog

Trying 31060000009999...Open

DIALOG INFORMATION SERVICES
PLEASE LOGON:
****
ENTER PASSWORD:
****
Welcome to DIALOG

Dialog level 02.18.00D

Last logoff: 05aug03 09:36:27
Logon file405 05aug03 10:22:39
HILIGHT set on as '*'
KWIC is set to 50.
* * * * See HELP NEWS 225 for information on new search prefixes and display codes

*** ***

SYSTEM:HOME
Cost is in DialUnits
Menu System II: D2 version 1.7.9 term=ASCII
*** DIALOG HOMEBASE(SM) Main Menu ***
Information:
1. Announcements (new files, reloads, etc.)
2. Database, Rates, & Command Descriptions
3. Help in Choosing Databases for Your Topic
4. Customer Services (telephone assistance, training, seminars, etc.) 5. Product Descriptions

Connections:
6. DIALOG(R) Document Delivery
7. Data Star(R)

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/H = Help /L = Logoff /NOMENU = Command Mode

Enter an option number to view information or to connect to an online service. Enter a BEGIN command plus a file number to search a database (e.g., B1 for ERIC).
? b 410

05aug03 10:22:40 User217743 Session D615.1
$0.00 0.161 DialUnits FileHomeBase
$0.00 Estimated cost FileHomeBase
$0.00 Estimated cost this search
$0.00 Estimated total session cost 0.161 DialUnits
File 410:Chronolog(R) 1981-2003/Aug
(c) 2003 The Dialog Corporation

Set Items Description
--- ----- -----------
? set hi *;set hi *
HILIGHT set on as '*'*
HILIGHT set on as ''
? b 411
05aug03 10:22:47 User217743 Session D615.2
$0.00 0.071 DialUnits File410
$0.00 Estimated cost File410
$0.03 TELNET
$0.03 Estimated cost this search
$0.03 Estimated total session cost 0.232 DialUnits
File 411:DIALINDEX(R)

DIALINDEX(R)
(c) 2003 The Dialog Corporation plc

*** DIALINDEX search results display in an abbreviated *** *** format unless you enter the SET DETAIL ON command. *** ? set files biochem
>>> 162 is unauthorized
>>>1 of the specified files is not available
You have 21 files in your file list.
(To see banners, use SHOW FILES command)
? s pm$1 or mr16$1

Your SELECT statement is:
s pm$1 or mr16$1

Items File
----- ----

No files have one or more items; file list includes 21 files.
? s pm()1 or mr16()1

Your SELECT statement is:
s pm()1 or mr16()1

Items File
----- ----
310 5: Biosis Previews(R)_1969-2003/Jul W4
15 6: NTIS_1964-2003/Aug W1
259 34: SciSearch(R) Cited Ref Sci_1990-2003/Jul W4
4 40: Enviroline(R)_1975-2003/Jul
85 50: CAB Abstracts_1972-2003/Jun
64 65: Inside Conferences_1993-2003/Aug W1
96 71: ELSEVIER BIOBASE_1994-2003/Aug W1
219 73: EMBASE_1974-2003/Jul W4

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        27   94: JICST-EPlus_1985-2003/Jul W4
        15   98: General Sci
Abs/Full-Text_1984-2003/Jun          39  103: Energy
SciTec_1974-2003/Jul B2
         2  143: Biol. & Agric. Index_1983-2003/Jun
    135  144: Pascal_1973-2003/Jul W3
       246  155: MEDLINE(R)_1966-2003/Aug W1
        54  156: ToxFile_1965-2003/Jul W4
         3  172: EMBASE Alert_2003/Aug W1
        20  305: Analytical Abstracts_1980-2003/Jul
W2           1  370: Science_1996-1999/Jul W3
        43  399: CA SEARCH(R)_1967-2003/UD=13906
         8  434: SciSearch(R) Cited Ref
Sci_1974-1989/Dec
  20 files have one or more items; file list includes 21
files.
? rf
Your last SELECT statement was:
  S PM()1 OR MR16()1

Ref      Items  File
---      -----  ----
N1         310    5: Biosis Previews(R)_1969-2003/Jul W4
N2         259   34: SciSearch(R) Cited Ref
Sci_1990-2003/Jul W4 N3         246  155:
MEDLINE(R)_1966-2003/Aug W1
N4         219   73: EMBASE_1974-2003/Jul W4
N5         135  144: Pascal_1973-2003/Jul W3
N6          96   71: ELSEVIER BIOBASE_1994-2003/Aug
W1
N7          85   50: CAB Abstracts_1972-2003/Jun
N8          64   65: Inside Conferences_1993-2003/Aug
W1 N9          54  156: ToxFile_1965-2003/Jul W4
N10         43  399: CA
SEARCH(R)_1967-2003/UD=13906
  20 files have one or more items; file list includes 21
files.
      - Enter P or PAGE for more -
? b n3,n1
     05aug03 10:24:27 User217743 Session D615.3
        $4.41   2.206 DialUnits File411
    $4.41 Estimated cost File411
    $0.46 TELNET
    $4.87 Estimated cost this search
    $4.90 Estimated total session cost   2.438 DialUnits
SYSTEM:OS - DIALOG OneSearch
 File 155:MEDLINE(R) 1966-2003/Aug W1
      (c) format only 2003 The Dialog Corp.
*File 155: Medline has been reloaded and accession
numbers have changed. Please see HELP NEWS 155.
 File   5:Biosis Previews(R) 1969-2003/Jul W4
      (c) 2003 BIOSIS

    Set  Items  Description
    ---  -----  -----------
? s pm()1 or mr16()1
       30000  PM
     5957076  1
         545  PM(W)1
          13  MR16
     5957076  1
          11  MR16(W)1
    S1    556  PM()1 OR MR16()1
? s pm()1
       30000  PM
     5957076  1
    S2    545  PM()1
? s mr16()1
          13  MR16
     5957076  1
    S3     11  MR16()1
? s s2 and antibody
         545  S2
      748137  ANTIBODY
    S4     54  S2 AND ANTIBODY
? s s4 and py>1999
          54  S4
     3689127  PY>1999
    S5      7  S4 AND PY>1999
? s s4 not s5
          54  S4
           7  S5
    S6     47  S4 NOT S5
? t s6/3,ab/all

 6/3,AB/1    (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.

11536284  98427561  PMID: 9756130
   IL-6 functions in cynomolgus monkeys blocked by
a humanized *antibody* to human IL-6 receptor.
 Imazeki I; Saito H; Hasegawa M; Shinkura H; Kishimoto
T; Ohsugi Y  Fuji-Gotemba Research Laboratories,
Chugai Pharmaceutical Co., Ltd, Tokyo, Japan.
 International journal of immunopharmacology
(ENGLAND)  Jul 1998, 20 (7) p345-57, ISSN
0192-0561  Journal Code: 7904799
 Document type: Journal Article
 Languages: ENGLISH
 Main Citation Owner: NLM
 Record type: Completed
 A humanized *antibody* to the human interleukin-6
receptor (IL-6R), hPM-1, blocked the interleukin-6
(IL-6) functions in normal cynomolgus monkey
lymphocytes in vitro. The binding activity of hPM-1 to
non-human primate IL-6R was examined in peripheral
blood lymphocytes by flow cytometry. *PM*-*1*
recognized the IL-6R on T lymphocytes of cynomolgus
and rhesus monkeys, but did not on those of
marmosets. The homology between human IL-6R and
its cynomolgus monkey counterpart was 97.3% in the
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extracellular domain of the amino acid sequence, as determined by DNA sequencing of the PCR product from peripheral blood mononuclear cells. *PM*-*1* inhibited two functional parameters in vitro in cynomolgus monkeys: (1), T-cell proliferation stimulated by phytohemaglutinin and human IL-6; (2), Immunoglobulin G-production evoked by Staphylococcus aureus Cowan-1- and human IL-6-stimulated B lymphocytes. These data show that hPM-1 binds to and functionally blocks the cynomolgus monkey IL-6 receptors.

6/3,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.

11314854 98194294 PMID: 9533100
Pasteurella challenge and ELISA serology evaluation of broiler breeders vaccinated with live cholera vaccine.
Sander J E; Resurreccion R S; Waltman W D; McMurray B L
Department of Avian Medicine, College of Veterinary Medicine, University of Georgia, Athens 30602-4875, USA.
Avian diseases (UNITED STATES) Jan-Mar 1998, 42 (1) p190-3, ISSN 0005-2086 Journal Code: 0370617
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Broiler breeder pullets were vaccinated against fowl cholera at 10 and 20 wk of age using a live *PM*-*1* Pasteurella multocida vaccine administered by wing web stick. *Antibody* production for P. multocida was measured at vaccination and at 1-4-wk intervals following vaccination by enzyme-linked immunosorbent assay. Groups of vaccinated birds were challenged at 23 and 32 wk of age. Two doses of a live *PM*-*1* P. multocida vaccine protected broiler breeder hens against virulent challenge up to 32 wk of age when measured *antibody* levels had a range of 1951-4346 and a geometric titer of 3000.

6/3,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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10991825 97345027 PMID: 9201394
Comparison of live avirulent *PM*-*1* and CU fowl cholera vaccines in turkeys.
Hopkins B A; Olson L D
Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Missouri, Columbia 65211, USA.
Avian diseases (UNITED STATES) Apr-Jun 1997, 41 (2) p317-25, ISSN 0005-2086 Journal Code: 0370617
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The live avirulent *PM*-*1* Pasteurella multocida vaccine, grown in brain-heart infusion broth, was evaluated and compared in two experiments with the Clemson University (CU) vaccine, which had been shown to be effective in preventing fowl cholera in turkeys. Experiment 1 was performed during warm environmental temperatures and Expt. 2 during cooler environmental temperatures. The *PM*-*1* vaccine was comparable with the CU vaccine in protecting turkeys against challenge with virulent P. multocida but was considered no less virulent than the CU because turkeys died after vaccination with both the *PM*-*1* and the CU vaccines. A significantly ($P < 0.05$) higher percentage of unvaccinated turkeys challenged during the cooler environmental temperatures died than did unvaccinated turkeys challenged during the warmer temperatures. A microtiter agglutination test demonstrated a significant ($P < 0.01$) correlation between the level of serum anti-P. multocida *antibody* found 1 wk after vaccination and survival after challenge with virulent P. multocida in Expt. 1 and a significant ($P < 0.05$) correlation between these parameters in Expt. 2. However, there was a significant ($P < 0.01$) negative correlation between serum anti-P. multocida *antibody* titer 1 wk after vaccination and body weight gained 4 wk after vaccination, but before challenge, in Expt. 1, suggesting that vaccination with the live vaccines may have had a negative effect on body weight gain. At 4 wk after challenge or 8 wk after vaccination in Expt. 2, there was also a highly significant ($P < 0.001$) negative correlation between these parameters in the surviving turkeys.

6/3,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.

10968410 97321108 PMID: 9177839
Single-chain Fv/folate conjugates mediate efficient lysis of folate-receptor-positive tumor cells.
Cho B K; Roy E J; Patrick T A; Kranz D M
Department of Biochemistry, University of Illinois, Urbana 61801-3792, USA.
Bioconjugate chemistry (UNITED STATES) May-Jun 1997, 8 (3) p338-46, ISSN 1043-1802 Journal Code: 9010319
Contract/Grant No.: AI35990; AI; NIAID; MH11189-01; MH; NIMH; RR07141; RR ; NCRR
Document type: Journal Article

Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Bispecific antibodies that bind to a tumor antigen and the T cell receptor (TCR) redirect cytotoxic T lymphocytes (CTL) to lyse tumor cells which have escaped normal immune recognition mechanisms. One well-characterized tumor antigen, the folate receptor (FR), is expressed on most ovarian carcinomas and some types of brain cancer. Recently, it was shown that conjugates of folate and anti-TCR antibodies are extremely potent bispecific agents that target tumor cells expressing the high-affinity folate receptor, but not normal cells expressing only the reduced folate carrier protein. In this paper, it is shown that the size of these conjugates can be reduced to the smallest bispecific agent yet described (30 kDa) by attaching folate to a single-chain *antibody*, scFv, of the anti-TCR *antibody* KJ16. The scFv/folate conjugates are as effective as IgG/folate conjugates in mediating lysis of FR4 tumor cells by CTL. The optimal folate density was in the range of 5-15 folate molecules per scFv or IgG molecule, which yielded half-maximal lysis values (EC50) of approximately 40 *pM* (*1*.2 ng/mL for scFv). Finally, the scFv/folate conjugates could efficiently target tumor cells even in the presence of free folic acid at concentrations that are normally found in serum. Compared to conventional bispecific antibodies, the small size of scFv/folate conjugates may prove advantageous in the ability to penetrate tumors and in reduced immunogenicity.

6/3,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.

10395703 96201129 PMID: 8619626
Purification and characterization of salivary kallikrein from an insectivore (Scalopus aquaticus): substrate specificities, immunoreactivity, and kinetic analyses.
Richards G P; Zintz C; Chao J; Chao L
National Marine Fisheries Service, Southeast Fisheries Science Center, Charleston Laboratory, South Carolina 29422-2607, USA.
Archives of biochemistry and biophysics (UNITED STATES) May 1 1996, 329 (1) p104-12, ISSN 0003-9861 Journal Code: 0372430 Contract/Grant No.: DE 09731; DE; NIDCR; HL 29397; HL; NHLBI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We report the successful one-step separation of tissue kallikrein from the salivary glands of an insectivore, the Eastern Atlantic mole (Scalopus aquaticus) by perfusion chromatography. Purified mole salivary kallikrein was characterized as a 30-kDa serine proteinase with a pI of 5.3 and a pH optimum of 9.0. It was readily recognized by human tissue kallikrein *antibody* in immunoblot analyses. It preferentially hydrolyzes fluorogenic peptidyl substrates with arginyl residues, rather than lysyl residues at the P1 substrate recognition site, indicating that it is like other mammalian kallikreins. Mole kallikrein efficiently releases kinin from low molecular weight human, dog, and bovine kininogen substrates with specific activities similar to that of human tissue kallikrein. Steady state kinetics performed with the synthetic tripeptidyl substrates, Phe-Phe-Arg-, Pro-Phe-Arg, and Val-Leu-Arg-7-amino-4-methylcoumarin, gave K(m) values for mole kallikrein of 3.3, 46.1, and 2.8 microM, respectively, and specificity constants, kcat/K(m), of 3818, 165, and 8714 s-1 *pM*- *1* , respectively. Mole kallikrein, when compared with human and rat tissue kallikreins, more closely resembles human kallikrein based on immunoreactivity and kininogenase activity. Mole kallikrein appears to be a member of a single gene or small multigene family. S. aquaticus is recommended for studying the evolution of mammalian proteins and may offer advantages over rodent models for biomedical research.

6/3,AB/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.

08626111 95314638 PMID: 7540841
The regulatory site of functional GTP binding protein coupled to the high affinity cholecystokinin receptor and phospholipase A2 pathway is on the G beta subunit of Gq protein in pancreatic acini.
Tsunoda Y; Owyang C
Department of Internal Medicine, University of Michigan, Ann Arbor 48109, USA.
Biochemical and biophysical research communications (UNITED STATES) Jun 15 1995, 211 (2) p648-55, ISSN 0006-291X Journal Code: 0372516 Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
A non-hydrolysable guanosine nucleotide analog, GTP[S] at 200 microM, stimulated amylase secretion which was inhibited by an anti-phospholipase A2 (PLA2) *antibody* in permeabilized pancreatic acini, indicating that the PLA2 pathway is linked to the GTP binding protein. A high affinity cholecystokinin (CCK) receptor agonist, CCK-OPE (10 microM), and a low affinity receptor agonist, CCK-8 (0.1 microM), both caused

amylase secretion in permeabilized cells. The action of CCK-OPE was abolished by the G beta *antibody* but not by the G alpha-q,11 *antibody*, whereas the opposite was true of the CCK-8 response. Biscoclaurine alkaloid isotetrandrine (10 microM), a specific inhibitor of PLA2-coupled G proteins, abolished Ca2+ oscillations and amylase secretion induced by CCK-OPE (0.1-100 nM), but not by CCK-8 (10 pM) in intact acini. Gp antagonist-2A (10 microM), which inhibits the activation of Gq, also inhibited the actions of CCK-OPE (10 *pM*-*1* microM) in intact acini. These observations indicate that the functional unit of the heterotrimeric G protein coupled to the high affinity CCK receptor appears to be different from that linked to the low affinity CCK receptor/Gq-alpha pathway. The regulatory site of this G protein coupled to the high affinity CCK receptor is on the beta subunit of Gq protein which elicits Ca2+ oscillations and monophasic amylase secretion via the PLA2 pathway.

6/3,AB/7 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08269056 94335117 PMID: 8057475
Cryptic nature of envelope V3 region epitopes protects primary monocytotropic human immunodeficiency virus type 1 from *antibody* neutralization.
Bou-Habib D C; Roderiquez G; Oravecz T; Berman P W; Lusso P; Norcross M A Division of Hematologic Products, Food and Drug Administration, Bethesda, Maryland 20892.
Journal of virology (UNITED STATES) Sep 1994, 68 (9) p6006-13, ISSN 0022-538X Journal Code: 0113724
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Characterization of biological and immunological properties of human immunodeficiency virus type 1 (HIV-1) is critical to developing effective therapies and vaccines for AIDS. With the use of a novel CD4+ T-cell line (*PM*-*1*) permissive to infection by both monocytotropic (MT) and T-cell-tropic virus types, we present a comparative analysis of the immunological properties of a prototypic primary MT isolate of HIV-1 strain JR-CSF (MT-CSF) with those of a T-cell-tropic variant (T-CSF) of the same virus, which emerged spontaneously in vitro. The parental MT-CSF infected only *PM*-*1* cells and was markedly resistant to neutralization by sera from HIV-1-infected individuals, rabbit antiserum to recombinant MT-CSF gp120, and anti-V3 monoclonal antibodies. The T-CSF variant infected a variety of CD4+ T-cell lines, contained positively charged amino acid substitutions in the gp120 V3 region, and was highly sensitive to *antibody* neutralization. Neutralization and *antibody* staining of T-CSF-expressing cells were significantly inhibited by HIV-1 V3 peptides; in contrast, the MT strain showed only weak V3-specific binding of polyclonal and monoclonal antibodies. Exposure of *PM*-*1* cells to a mixture of both viruses in the presence of human anti-HIV-1 neutralizing antiserum resulted in infection with only MT-CSF. These results demonstrate that although the V3 region of MT viruses is immunogenic, the target epitopes in the V3 principal neutralizing domain on the membrane form of the MT envelope appear to be cryptic or hidden from blocking antibodies.

6/3,AB/8 (Item 8 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08152399 94218274 PMID: 8165163
Direct and correlated responses to multitrait, divergent selection for immunocompetence.
Kean R P; Cahaner A; Freeman A E; Lamont S J
Department of Animal Science, Iowa State University, Ames 50011. Poultry science (UNITED STATES) Jan 1994, 73 (1) p18-32, ISSN 0032-5791 Journal Code: 0401150
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Leghorn lines had been selected for an immunocompetence index based on four traits: *antibody* production to Mycoplasma gallisepticum (MG) and Pasteurella multocida (PM) vaccines, reticuloendothelial clearance of colloidal carbon (CCA), and cell-mediated, wing web response to phytohemagglutinin (PHA). The purpose of this study was to produce replicated lines of chickens with divergent levels of multitrait immunocompetence by index selection. The objectives of analyses of Generations 5 to 7 of this study was to characterize these lines with respect to immune-response traits, correlations among these traits, and correlated responses in other important production traits. Differences ($P < .05$) existed between the lines selected for high or low immune response and between the two replicates in mean breeding values and in individual immune-response traits. Averages of heritability estimates, weighted by number of offspring and pooled across three generations (two cycles of selection), estimated by using sire variance components and parent-offspring correlations were, respectively, .16 and .09 for the index, .31 and .08 for MG, .21 and -.02 for PM, .06 and .05 for CCA, and .08 and .12 for PHA. Realized heritabilities (response divided by effective selection differential) pooled across the two

selection cycles, were .19 and .11 for the index, .06 and -.01 for MG, .44 and .32 for *PM*, *1*.52 and -1.21 for CCA, and .48 and .15 for PHA, for Replicates 1 and 2, respectively. Phenotypic correlations among traits were generally small, and several estimates were negative. Estimates of genetic correlation varied widely. Juvenile and adult body weights, age of first egg, 32-wk egg weight, and rate of egg production were analyzed to evaluate effects of selection on these traits of direct economic importance. Very few differences were noted.

6/3,AB/9 (Item 9 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08007527 94073246 PMID: 8252089
[Melatonin secretion in Klinefelter's syndrome]
Studio della secrezione di melatonina nella sindrome di Klinefelter. Grugni G; Carani C; Granata A; Guzzaloni G; Ardizzi A; Morabito F Centro Auxologico Italiano di Piancavallo-IRCCS-Dipartimento di Endocrinologia-Universita di Modena.
Archivio italiano di urologia, andrologia - organo ufficiale di Societa italiana di ecografia urologica e nefrologica / Associazione ricerche in urologia (ITALY) Oct 1993, 65 (5) p571-5, ISSN 1124-3562 Journal Code: 9308247
Document type: Journal Article ; English Abstract
Languages: ITALIAN
Main Citation Owner: NLM
Record type: Completed
It has been observed that the pineal gland seems to modulate diencephalic neuroendocrine activity through its principal hormone, melatonin. In animals, melatonin inhibits the secretion and release of hypophyseal gonadotropins, probably by inhibiting hypothalamic releasing factors; in man, on the contrary, the administration of LHRH seems to have a stimulating effect on melatonin serum levels. In the light of this, in pathologies characterised by an imbalance in the hypothalamus-hypophyseal-gonad axis, it is possible to hypothesise variations in the secretion of melatonin and/or in its circadian fluctuations. In order to clarify further the relationship between the epiphysis and the hypothalamus-hypophyseal axis, the present study evaluated the pattern of melatonin secretion in a group of 16 patients with Klinefelter's syndrome. The circadian rhythm of melatonin secretion was determined from venous blood samples taken at 9 am, 1 pm, 5 pm, 9 *pm*, *1* am and 5 am; the same protocol was also followed in two control groups of respectively prepuberal and puberal healthy subjects. During the night samples were taken as rapidly as possible, using a red light source in order to not interfere with melatonin secretion. All of the examinations were performed during the winter period. Serum levels of melatonin were determined, after extractions with diethylether, by means of a double *antibody* RIA using commercially available kits (Bioscience Product--The Netherlands). Intra- and inter-assay coefficients of variation were respectively 3% and 8%. The data are reported as mean values +/- SD; the results were analysed by means of Student's test for unpaired data and analysis of variance.(ABSTRACT TRUNCATED AT 250 WORDS)

6/3,AB/10 (Item 10 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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07698534 93153784 PMID: 8428365
Reshaping a human *antibody* to inhibit the interleukin 6-dependent tumor cell growth.
Sato K; Tsuchiya M; Saldanha J; Koishihara Y; Ohsugi Y; Kishimoto T; Bendig M M
Fuji-Gotemba Research Laboratories, Chugai Pharmaceutical, Co., Ltd., Shizuoka, Japan.
Cancer research (UNITED STATES) Feb 15 1993, 53 (4) p851-6, ISSN 0008-5472 Journal Code: 2984705R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The mouse *PM*-*1* monoclonal *antibody* binds to the human interleukin 6 receptor, inhibits IL-6 functions, and shows strong antitumor cell activity against multiple myeloma cells. In order to be effective as a therapeutic agent administered to human patients in repeated doses, reshaped human *PM*-*1* antibodies consisting of human REI-based light chain and NEW-based heavy chain variable regions were designed and constructed with the assistance of a structural model of the mouse *PM*-*1* variable regions. The best reshaped human *PM*-*1* *antibody* is equivalent to mouse or chimeric *PM*-*1* *antibody* in terms of antigen binding and growth inhibition against multiple myeloma cells. Only a few minor changes in the human framework regions were required to recreate the mouse *PM*-*1* antigen-binding site within a human *antibody*. The reshaped human *PM*-*1* *antibody* , therefore, could be efficacious in human multiple myeloma patients.

6/3,AB/11 (Item 11 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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07228898 92091431 PMID: 1727819
Trophoblast-derived tumor necrosis factor-alpha

induces release of human chorionic gonadotropin using interleukin-6 (IL-6) and IL-6-receptor-dependent system in the normal human trophoblasts. Li Y; Matsuzaki N; Masuhiro K; Kameda T; Taniguchi T; Saji F; Yone K; Tanizawa O

Department of Obstetrics and Gynecology, Osaka University Medical School, Japan.

Journal of clinical endocrinology and metabolism (UNITED STATES) Jan 1992, 74 (1) p184-91, ISSN 0021-972X Journal Code: 0375362 Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The titer of tumor necrosis factor-alpha (TNF alpha) secreted by placental blocks was determined by enzyme immunoassay. The source of placental TNF alpha was immunohistochemically demonstrated with monoclonal anti-TNF alpha *antibody* to be only trophoblasts. Purified trophoblasts produced 174.4 ng/L TNF alpha by 24 h of culture in vitro. To investigate the role of TNF alpha in placental hormonogenesis, purified trophoblasts were stimulated with recombinant TNF alpha (rTNF alpha) to determine the hCG titer by enzyme immunoassay. Trophoblasts stimulated with rTNF alpha released hCG in a dose-dependent fashion with kinetics similar to those of recombinant interleukin-1 (rIL-1)-stimulated trophoblasts. The stimulated trophoblasts released IL-6 before hCG, but failed to show hCG release when pretreated with anti-IL-6 receptor (anti-IL-6-R) monoclonal *antibody* *PM*-*1*. However, the pretreatment of trophoblasts with *PM*-*1* did not interfere with rTNF alpha-induced IL-6 release, ruling out the possibility of a nonspecific toxic effect of *PM*-*1* on trophoblasts. These results suggest that trophoblast-derived TNF alpha induced IL-6 release and then activated the IL-6-R system in trophoblasts to release hCG. Since IL-1 has also been demonstrated to induce similar release of IL-6 and hCG from trophoblasts, the effects of TNF alpha and IL-1 on these trophoblast functions were also examined. Simultaneous stimulation of trophoblasts with rTNF alpha and gamma IL-1 alpha resulted in synergistic enhancement of IL-6 release, subsequently leading to enhanced hCG release. Collectively, trophoblast-derived TNF alpha and IL-1 synergistically regulated the level of IL-6 secreted by trophoblasts, the magnitude of which determined the level of hCG released by activating the IL-6-R system in trophoblasts.

6/3,AB/12 (Item 12 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06905648 91145927 PMID: 2149670

Paradoxical enhancement of interleukin-2-mediated cytotoxicity against K562 cells by addition of a low dose of methotrexate.

Nakarai T; Ueno Y; Ueno Y; Koizumi S

Department of Pediatrics, Kanazawa University School of Medicine, Japan. Cancer immunology, immunotherapy - CII (GERMANY) 1990, 32 (1) p8-12, ISSN 0340-7004 Journal Code: 8605732

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

In vitro effects of methotrexate (MTX) on interleukin-2(IL-2)-mediated cytotoxicity of peripheral blood mononuclear cells (PBMC) were studied. PBMC were incubated with human recombinant IL-2 (25 U/ml) for 72 h; during the last 24 h, various concentrations (10 *pM*-*1* microM) of MTX were added to the culture. Cytotoxicity against k562 cells was measured by a 4-h 51Cr-release assay. The IL-2-mediated cytotoxicity was paradoxically increased at around a concentration (10 nM) MTX. Such a low concentration of MTX showed no anti-proliferative effect on cell growth. This enhancement with 10 nM MTX was shown only in an E-rosette+ (E+) population, but not in E-rosette- (E-). In addition, when E+ cells were treated with an anti-CD16 monoclonal *antibody* plus complement after incubation with IL-2 and MTX, MTX-induced enhancement was lost, suggesting that an E+CD16+ cell population was mainly involved in this augmentation. Positively sorted E+CD16+ cells showed similar enhancement of cytotoxicity after treatment with IL-2 plus MTX. On the other hand, MTX treatment did not show the phenotypical changes including of the E+CD16+ cells, indicating that this treatment did not affect the differentiation and proliferation of the specific cell subset. Our results indicate that a low dose of MTX could have a role in the regulation of immunological anti-cancer surveillance systems through the natural killer and lymphokine-activated cytotoxic cells.

6/3,AB/13 (Item 13 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06712298 90338316 PMID: 2116439

Trophoblast-derived interleukin-6 (IL-6) regulates human chorionic gonadotropin release through IL-6 receptor on human trophoblasts. Nishino E; Matsuzaki N; Masuhiro K; Kameda T; Taniguchi T; Takagi T; Saji F; Tanizawa O

Department of Obstetrics and Gynecology, Osaka University Medical School, Japan.

Journal of clinical endocrinology and metabolism (UNITED STATES) Aug 1990, 71 (2) p436-41, ISSN

0021-972X Journal Code: 0375362 Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We examined the capacity of trophoblast-derived interleukin-6 (IL-6) to stimulate secretion of placental hormones, including hCG. IL-6 stimulated hCG secretion by trophoblasts to a level similar to that stimulated by a GnRH analog. The analog, however, released hCG by an IL-6-independent mechanism because *PM*-*1*, a monoclonal *antibody* specific for IL-6 receptors (R), failed to block GnRH-mediated responses, but completely blocked IL-6-mediated hCG secretion, suggesting the existence of two distinct regulatory pathways for hCG release. Immunohistochemical analysis with another IL-6-R-specific *antibody*, MT-18, showed that IL-6-R was located only on the trophoblast layer of the placenta. Our data revealed the existence of a local regulatory network by which trophoblast-derived IL-6 interacts with IL-6-R on the trophoblasts, resulting in hCG release. Thus, two different regulatory networks, an IL-6 and IL-6-R system and a GnRH and GnRH-R system, regulate hCG release by human trophoblasts independently.

6/3,AB/14 (Item 14 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06671461 90297235 PMID: 2360639
Molecular variants of cholecystokinin after endogenous stimulation in humans: a time study.
Eysselein V E; Eberlein G A; Hesse W H; Schaeffer M; Grandt D; Williams R ; Goebell H; Reeve J R
Department of Gastroenterology and Surgery, Harbor-University of California, Los Angeles, Medical Center, Torrance 90509.
American journal of physiology (UNITED STATES) Jun 1990, 258 (6 Pt 1) pG951-7, ISSN 0002-9513 Journal Code: 0370511
Contract/Grant No.: DK-17294; DK; NIDDK; DK-33850; DK; NIDDK; DK-36200; DK; NIDDK
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The time-dependent release of molecular variants of cholecystokinin (CCK) into the circulation was studied before and 1, 2, and 4 h after a test meal in six healthy volunteers. At each time period, 100 ml of blood were drawn in a manner to inhibit CCK degradation. Plasma was formed and CCK concentrated by Sep-Pak C18 cartridge chromatography. Molecular variants of CCK and gastrin were well separated from each other by high-performance liquid chromatography (HPLC). Molecular forms of CCK and gastrin were measured by radioimmunoassay using an *antibody* that requires the presence of the carboxyl-terminal phenylalanine amide for full recognition, implying that biologically active forms were detected. HPLC elution positions of gastrin forms were determined using a gastrin-specific *antibody* . Chromatographic separation of CCK from gastrin forms was complete, allowing separate integration of gastrin and CCK forms. Therefore no subtraction of gastrin-like immunoreactivity from CCK-like immunoreactivity (CCK-LI) was necessary and CCK-LI could be directly determined. Peaks of CCK-LI were integrated in the column eluates and the plasma concentrations were calculated. Total plasma CCK-LI rose from a value of 2.4 +/- 0.6 pM before the test meal to 6.4 +/- 0.8, 6.6 +/- 0.9, and 5.8 +/- 1.2 *pM* *1*, 2, and 4 h postprandially. The major molecular forms released into the circulation eluted on HPLC in the position of CCK-58 and CCK-39 (which coelutes with CCK-33). Minor amounts were detected in the position of CCK-8. There was no significant difference in the relative proportions of the molecular forms released at the different time periods.(ABSTRACT TRUNCATED AT 250 WORDS)

6/3,AB/15 (Item 15 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06011053 89025771 PMID: 2460095
Effect of interleukin 1 beta on transducing mechanisms in 235-1 clonal pituitary cells. Part II: Modulation of calcium fluxes.
Schettini G; Meucci O; Florio T; Scala G; Landolfi E; Grimaldi M Dept. of Pharmacology, II School of Medicine, Napoli, Italy. Biochemical and biophysical research communications (UNITED STATES) Sep 30 1988, 155 (3) p1097-104, ISSN 0006-291X Journal Code: 0372516 Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
In the present study we investigated the effect of the interleukin 1 beta on intracellular free calcium concentrations in 235-1 cell line both in basal conditions and after stimulation by the calcium channel activator maitotoxin. Interleukin 1 beta (from 0.01 pM to 10 nM) was unable to significantly affect basal cytosolic free calcium levels in acute conditions. The preincubation of these cells with interleukin 1 beta for 48h modulates maitotoxin stimulation of calcium fluxes without modifying basal intracellular free calcium levels. Low concentrations of interleukin 1 beta (0.01 *pM*, *1* pM) caused a marked reduction of intracellular

free calcium concentrations increase induced by maitotoxin while higher doses of the monokine potentiated maitotoxin stimulation of calcium fluxes. The specificity of interleukin 1 beta effect was tested by means of polyclonal anti-interleukin 1 beta *antibody* (titer 1:100) which significantly abolished the inhibitory effect of interleukin 1 beta on free cytosolic calcium levels. These results show that a long lasting interaction of interleukin 1 beta with its receptor is able to influence voltage-sensitive calcium channels activation induced by maitotoxin in 235-1 cells.

6/3,AB/16 (Item 16 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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05266628 86267793 PMID: 2425740
Small nuclear ribonucleoprotein antigens are absent from 10S translation inhibitory ribonucleoprotein but present in cytoplasmic messenger ribonucleoprotein and polysomes.
Boak A M; Kovacs S A; Agris P F; Chakraborty D; Sarkar S Archives of biochemistry and biophysics (UNITED STATES) Jul 1986, 248 (1) p89-100, ISSN 0003-9861 Journal Code: 0372430
Contract/Grant No.: 2593; PHS; AM 13238; AM; NIADDK; AM 20305; AM; NIADDK Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
A cytoplasmic 10S ribonucleoprotein particle (iRNP), which is isolated from chick embryonic muscle, is a potent inhibitor of mRNA translation in vitro and contains a 4S translation inhibitory RNA species (iRNA). The iRNP particle shows similarity in size to the small nuclear ribonucleoprotein (snRNP) particles. Certain autoimmune disease patients contain antibodies directed against snRNP antigenic determinants. The possibility that iRNP may be related to the small nuclear particles was tested by immunoreactivity with monospecific autoimmune antibodies to six antigenic determinants (Sm, RNP, *PM*-*1*, SS-A (Ro), SS-B (La), and Scl-70). By Ouchterlony immunodiffusion assays, the cytoplasmic 10S iRNP did not show any immunoreactivity. Also, a more sensitive hemagglutination inhibition assay for detecting Sm and RNP antigens failed to show reactivity with the 10S iRNP. Thus, the 10S iRNP particles are distinct from the similarly sized snRNP. However, free and polysomal messenger ribonucleoprotein (mRNP) particles and polysomes also isolated from chick embryonic muscle and analyzed by Ouchterlony immunodiffusion and hemagglutination inhibition for the presence of the antigenic determinants showed reactivity to Sm and RNP autoantibodies, but were not antigenic for the other four antibodies. Some of the Sm antigenic peptides of mRNP particles and polysomes were identical to those purified from calf thymus nuclear extract, as judged by Western blot analysis. The association of Sm with free and polysomal mRNP and polysomes suggests that Sm may be involved in some cytoplasmic aspects of mRNA metabolism, in addition to a nuclear function in mRNA processing.

6/3,AB/17 (Item 17 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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04840787 85147154 PMID: 6335633
[What can be expected of biological tests in scleroderma?] Que peut-on attendre des examens biologiques dans les sclerodermies? Meyer O
Annales de medecine interne (FRANCE) 1984, 135 (8) p584-90, ISSN 0003-410X Journal Code: 0171744
Document type: Journal Article ; English Abstract
Languages: FRENCH
Main Citation Owner: NLM
Record type: Completed
Biological investigations are useful from three main view points in systemic scleroderma: diagnosis: the demonstration of antinuclear antibodies which are quite specific for these conditions: antinuclear anti-ECT (nRNP, Scl 70, KU, *PM* *1*) and anticentromere antibodies; prognosis: the presence of some antibodies is associated with a more favourable outcome: anti-nRNP and Sharp's syndrome, anti-centromere and the CREST syndrome, anti-KU and syndromes overlapping with polymyositis; pathogenic: the study of abnormalities of T-lymphocyte function, immunogenetic (HLA grouping) studies, cytotoxic factors for vascular endothelium, mitogenic factors of fibroblasts and the "chromosome breaking" factor.

6/3,AB/18 (Item 18 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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04815712 85122015 PMID: 3871616
Immunogenetic studies of juvenile dermatomyositis. III. Study of *antibody* to organ-specific and nuclear antigens.
Pachman L M; Friedman J M; Maryjowski-Sweeney M L; Jonnason O; Radvany R M; Sharp G C; Cobb M A; Battles N D; Crowe W E; Fink C W; et al Arthritis and rheumatism (UNITED STATES) Feb 1985, 28 (2) p151-7, ISSN 0004-3591 Journal Code: 0370605
Contract/Grant No.: AN 21589; PHS
Document type: Journal Article
Languages: ENGLISH

Main Citation Owner: NLM
Record type: Completed
Ninety children with definite juvenile dermatomyositis (JDMS), who had been HLA typed, were tested for the presence of tissue or organ-specific antibodies. Sixty had active disease at the time of study. The mean disease duration was 4 years, and 30 had soft tissue calcifications. The following autoantibodies were sought: thyroid, gastric parietal cells, smooth muscle, striated muscle, microsomes, mitochondria, DNA, extractable nuclear antigen, Sm, *PM*-*1*, antinuclear *antibody* (ANA), and rheumatoid factor. Only the ANA and *PM*-*1* were more frequent in patients than in controls (P less than 0.0002 and P less than 0.001, respectively). Higher levels of immune complexes (P less than 0.01) were found in sera from patients with JDMS than in sera from controls and were correlated with the presence of ANA in patients (P less than 0.01). Soft tissue calcification was not associated with any autoantibody or HLA antigen, but with disease duration and activity (P less than 0.001 and P less than 0.05, respectively). There was no association between the occurrence of any autoantibody and the presence of HLA-B8 or DR3 among the white patients with JDMS. The frequency of autoantibodies in 43 full siblings of children with JDMS was not increased. We conclude that children with JDMS, with or without HLA-B8/DR3, do not show evidence of a generalized nonspecific *antibody* response to tissue antigens. The significance of the increased *antibody* to nuclear antigens ANA and *PM*-*1* remains to be determined.

6/3,AB/19 (Item 19 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

04776735 85082887 PMID: 6334745
Clinical relevance of *PM*-*1* *antibody* and physiochemical characterization of *PM*-*1* antigen.
Treadwell E L; Alspaugh M A; Wolfe J F; Sharp G C
Journal of rheumatology (CANADA) Oct 1984, 11 (5) p658-62, ISSN 0315-162X Journal Code: 7501984
Contract/Grant No.: AM 20305; AM; NIADDK
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Using an improved immunodiffusion test with partially purified antigen, *PM*-*1* *antibody* was identified in the serum of 18 patients. In 67% this system was associated with a polymyositis-scleroderma overlap, it occurred less frequently in polymyositis, dermatomyositis and scleroderma, and was not detected in other rheumatic diseases. The predominant clinical features of *PM*-*1* positive patients were muscle weakness, sclerodactyly, Raynaud's phenomenon and pulmonary disease; widespread sclerodermatous features with infrequent. Characterization of the *PM*-*1* antigen showed it to be a heat sensitive, trypsin sensitive acidic protein associated with the cell nucleus and possibly with nucleoli.

6/3,AB/20 (Item 20 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

04610284 84253638 PMID: 6377871
Standardization of the immunofluorescence test for autoantibody to nuclear antigens (ANA): use of reference sera of defined *antibody* specificity.
Molden D P; Nakamura R M; Tan E M
American journal of clinical pathology (UNITED STATES) Jul 1984, 82 (1) p57-66, ISSN 0002-9173 Journal Code: 0370470
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Standardization of the indirect immunofluorescence antinuclear *antibody* (IF-ANA) test can be improved for a given substrate with use of reference ANA sera, uniform assay conditions, and standardization of optical systems. To accomplish this, reference sera from the Arthritis Foundation with defined *antibody* specificities for nDNA, SS-B, RNP, Sm, nucleoli, and "speckled pattern" were reacted with commonly used IF-ANA substrates, mouse kidney sections, KB and HEp-2 tissue culture cells. Reagents and assay conditions used were those provided with the substrates in commercially available IF-ANA kits. A microscope slide with graded intensities of fluorescent beads was used to standardize microscope fluorescence intensity readings. The authors' fluorescence pattern and intensity results should be directly comparable to results obtained in other laboratories for the six antibodies for which reference sera are available. Although no defined sera are widely available for SS-A, Scl-70, *PM*-*1* , and centromere antibodies, the ability of each substrate to detect these antinuclear antibodies as well as mitochondrial, smooth muscle, ribosomal and microsomal antibodies also was tested. HEp-2 cells and KB cells were found to be superior to mouse kidney sections for detection of SS-A, Scl-70, *PM*-*1* and centromere antinuclear antibodies. Mouse kidney sections were superior for screening of sera for the absence of ANA as well as for detection of smooth muscle and liver-kidney microsomal antibodies. Other antibodies were detected with equal sensitivity with all substrates and each of the three ANA kits used in the study performed

satisfactorily. Use of reference sera as well as optical standardization is recommended for IF-ANA testing.

6/3,AB/21 (Item 21 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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04055048 83184276 PMID: 6341205
Autoantibodies to nonhistone nuclear antigens and their clinical significance.
Nakamura R M; Tan E M
Human pathology (UNITED STATES) May 1983, 14 (5) p392-400, ISSN 0046-8177 Journal Code: 9421547
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Identification of the immunologic specificity of antinuclear antibodies (ANAs) in the various systemic rheumatic diseases has become increasingly important. The standard immunofluorescence technique may enable detection of antibodies to nuclear antigens present in abundance in the nucleus, such as DNA, histones, Sm, nuclear ribonucleoprotein (nRNP), and SS-B/La. The nuclear antigens present in low concentrations, such as SS-A, proliferating cell nuclear antigen (PCNA), rheumatoid arthritis nuclear antigen (RANA), and Ku antigens, are unique to cell types, and their detection requires special substrates or reagent systems. Anti-Sm, anti-Scl-70, anticentromere, and anti-*PM*-*1* are characteristic serologic markers for systemic lupus erythematosus, scleroderma, the CREST syndrome of scleroderma, and polymyositis, respectively. Distinct profiles of ANA characterize different rheumatic diseases. A number of ANAs are found in SLE, whereas other diseases are characterized by the presence or absence of a certain ANA or by differences in mean ANA titers. Specific ANAs have been used to isolate and characterize nuclear antigens at molecular and functional levels.

6/3,AB/22 (Item 22 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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03971926 83100551 PMID: 7181969
Assessment of the immune responsiveness of mice irradiated with continuous wave or pulse-modulated 425-MHz radio frequency radiation. Smialowicz R J; Riddle M M; Weil C M; Brugnolotti P L; Kinn J B
Bioelectromagnetics (UNITED STATES) 1982, 3 (4) p467-70, ISSN 0197-8462 Journal Code: 8008281
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Groups of female BALB/C mice were irradiated with 425-MHz radio frequency (RF) radiation either continuous wave (CW) or pulse modulated (*PM*, *1* -ms pulse width, 250 pulses/s). Mice were irradiated in a rectangular strip-transmission line at average forward powers of 78, 17.7, or 5 W for CW and 17.7, 5, or 1.25 W for PM. The mean specific absorption rate, as measured using twin-well calorimetry was 7.7 W/kg for a forward power of 70 W. No differences in the mitogen-stimulated response of lymphocytes or in the primary *antibody* response to sheep erythrocytes or polyvinylpyrrolidone were observed between irradiated and sham-irradiated mice, nor between mice exposed to either CW or PM 425-MHz RF radiation.

6/3,AB/23 (Item 23 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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03804777 82216603 PMID: 6979627
The *PM*-*1* *antibody* test in a patient with rheumatic complaints.
Solomon S D
Journal of rheumatology (CANADA) Jan-Feb 1982, 9 (1) p157-8, ISSN 0315-162X Journal Code: 7501984
Document type: Letter
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

6/3,AB/24 (Item 24 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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02384322 77071762 PMID: 318657
Antinuclear *antibody* with distinct specificity for polymyositis. Wolfe J F; Adelstein E; Sharp G C
Journal of clinical investigation (UNITED STATES) Jan 1977, 59 (1) p176-8, ISSN 0021-9738 Journal Code: 7802877
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
In the course of studying antinuclear antibodies in the rheumatic diseases, a new precipitin reaction (provisionally referred to as *PM*- *1*) was observed between calf thymus nuclear extract and polymyositis sera. Objectives of this study were to further define the immunologic nature of this reaction and to determine its specificity for polymyositis. Immunodiffusion studies using calf thymus nuclear extract revealed the *PM*-*1* precipitin line in 17 of 28 patients with

polymyositis. This reaction was not produced by sera of 460 patients with other diseases. Enzyme and heat treatments of the nuclear extract showed that *PM*-*1* was distinct from native DNA, ribonucleoprotein, and Sm antigens. Fractionation of *PM*-*1*-positive serum by 30% ammonium sulphate and Sephadex G-200 chromatography revealed that the factor producing the *PM*-*1* precipitin reaction was in a serum fraction which showed only IgG by immunoelectrphoresis against anti-whole human serum. Because of the apparent strong specificity, the *PM*-*1* system may represent a marker *antibody* for polymyositis.

6/3,AB/25 (Item 25 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

01350934 72229368 PMID: 4339414
Rescue of the genome of the defective murine sarcoma virus from a non-producer hamster tumor cell line, *PM*-*1*, with murine and feline leukemia viruses as helpers.
Monti-Bragadin C; Ulrich K
International journal of cancer. Journal international du cancer (DENMARK) Mar 15 1972, 9 (2) p383-92, ISSN 0020-7136 Journal Code: 0042124 Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

6/3,AB/26 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11667271 BIOSIS NO.: 199800449002
IL-6 functions in cynomolgus monkeys blocked by a humanized *antibody* to human IL-6 receptor.
AUTHOR: Imazeki Ikuo(a); Saito Hiroyuki; Hasegawa Masakazu; Shinkura Hirofumi; Kishimoto Tadamitsu; Ohsugi Yoshiyuki
AUTHOR ADDRESS: (a)Fuji-Gotemba Res. Lab., Chugai Pharm. Co. Ltd., 3-41-8 Takada, Toshima-ku, Tokyo 171**Japan
JOURNAL: International Journal of Immunopharmacology 20 (7):p345-357 July, 1998
ISSN: 0192-0561
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A humanized *antibody* to the human interleukin-6 receptor (IL-6R), hPM-1, blocked the interleukin-6 (IL-6) functions in normal cynomolgus monkey lymphocytes in vitro. The binding activity of hPM-1 to nonhuman primate IL-6R was examined in peripheral blood lymphocytes by flow cytometry. *PM*-*1* recognized the IL-6R on T lymphocytes of cynomolgus and rhesus monkeys, but did not on those of marmosets. The homology between human IL-6R and its cynomolgus monkey counterpart was 97.3% in the extracellular domain of the amino acid sequence, as determined by DNA sequencing of the PCR product from peripheral blood mononuclear cells. *PM*-*1* inhibited two functional parameters in vitro in cynomolgus monkeys: (1), T-cell proliferation stimulated by phytohemaglutinin and human IL-6; (2), Immunoglobulin G-production evoked by Staphylococcus aureus Cowan-1- and human IL-6-stimulated B lymphocytes. These data show that hPM-1 binds to and functionally blocks the cynomolgus monkey IL-6 receptors.

1998

6/3,AB/27 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11436074 BIOSIS NO.: 199800217406
Pasteurella challenge and ELISA serology evaluation of broiler breeders vaccinated with live cholera vaccine.
AUTHOR: Sander Jean E(a); Resurreccion Reynaldo S; Waltman W Douglas; McMurray Birch L
AUTHOR ADDRESS: (a)Dep. Avian Med., Coll. Vet. Med., Univ. Georgia, Athens, GA 30602-4875**USA
JOURNAL: Avian Diseases 42 (1):p190-193 Jan.-March, 1998
ISSN: 0005-2086
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English; Spanish

ABSTRACT: Broiler breeder pullers were vaccinated against fowl cholera at 10 and 20 wk of age using a live *PM*-*1* Pasteurella multocida vaccine administered by wing web stick. *Antibody* production for P. multocida was measured at vaccination and at 1-4-wk intervals following vaccination by enzyme-linked immunosorbent assay. Groups of vaccinated birds were challenged at 23 and 32 wk of age. Two doses of a live *PM*-*1* P. multocida vaccine protected broiler breeder hens against virulent challenge up to 32 wk of age when measured *antibody* levels had a range of 1951-4346 and a geometric titer of 3000.

1998

6/3,AB/28 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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11023608 BIOSIS NO.: 199799644753
Comparison of live avirulent *PM*-*1* and CU fowl cholera vaccines in turkeys.
AUTHOR: Hopkins Brett A; Olson Leroy D
AUTHOR ADDRESS: Dep. Vet. Pathobiol., Coll. Vet. Med., Univ. Mo., Columbia, MO 65211**USA
JOURNAL: Avian Diseases 41 (2):p317-325 1997
ISSN: 0005-2086
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English; Spanish

ABSTRACT: The live avirulent *PM*-*1* Pasteurella multocida vaccine, grown in brain-heart infusion broth, was evaluated and compared in two experiments with the Clemson University (CU) vaccine, which had been shown to be effective in preventing fowl cholera in turkeys. Experiment 1 was performed during warm environmental temperatures and Expt. 2 during cooler environmental temperatures. The *PM*-*1* vaccine was comparable with the CU vaccine in protecting turkeys against challenge with virulent P. multocida but was considered no less virulent than the CU because turkeys died after vaccination with both the *PM* -*1* and the CU vaccines. A significantly (P lt 0.05) higher percentage of unvaccinated turkeys challenged during the cooler environmental temperatures died than did unvaccinated turkeys challenged during the warmer temperatures. A microtiter agglutination test demonstrated a significant (P lt 0.01) correlation between the level of serum anti-P. multocida *antibody* found 1 wk after vaccination and survival after challenge with virulent P. multocida in Expt. 1 and a significant (P lt 0.05) correlation between these parameters in Expt. 2. However, there was a significant (P lt 0.01) negative correlation between serum anti-P. multocida *antibody* titer 1 wk after vaccination and body weight gained 4 wk after vaccination, but before challenge, in Expt. 1, suggesting that vaccination with the live vaccines may have had a negative effect on body weight gain. At 4 wk after challenge or 8 wk after vaccination in Expt. 2, there was also a highly significant (P lt 0.001) negative correlation between these parameters in the surviving turkeys.

1997

6/3,AB/29 (Item 4 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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10990232 BIOSIS NO.: 199799611377
Single-chain Fv/folate conjugates mediate efficient lysis of folate-receptor-positive tumor cells.
AUTHOR: Cho Bryan K; Roy Edward J; Patrick Todd A; Kranz David M(a) AUTHOR ADDRESS: (a)Dep. Biochemistry, Univ. Illinois, 600 South Matthews Ave., Urbana, IL 61801-3792**USA
JOURNAL: Bioconjugate Chemistry 8 (3):p338-346 1997
ISSN: 1043-1802
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Bispecific antibodies that bind to a tumor antigen and the T cell receptor (TCR) redirect cytotoxic T lymphocytes (CTL) to lyse tumor cells which have escaped normal immune recognition mechanisms. One well-characterized tumor antigen, the folate receptor (FR), is expressed on most ovarian carcinomas and some types of brain cancer. Recently, it was shown that conjugates of folate and anti-TCR antibodies are extremely potent bispecific agents that target tumor cells expressing the high-affinity folate receptor, but not normal cells expressing only the reduced folate carrier protein. In this paper, it is shown that the size of these conjugates can be reduced to the smallest bispecific agent yet described (30 kDa) by attaching folate to a single-chain *antibody*, scFv, of the anti-TCR *antibody* KJ16. The scFv/folate conjugates are as effective as IgG/folate conjugates in mediating lysis of FRI tumor cells by CTL. The optimal folate density was in the range of 5-15 folate molecules per scFv or IgG molecule, which yielded half-maximal lysis values (EC50) of approximately 40 *pM* (*1*.2 ng/mL for scFv). Finally, the scFv/folate conjugates could efficiently target tumor cells even in the presence of free folic acid at concentrations that are normally found in serum. Compared to conventional bispecific antibodies, the small size of scFv/folate conjugates may prove advantageous in the ability to penetrate tumors and in reduced immunogenicity.
1997

6/3,AB/30 (Item 5 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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10367853 BIOSIS NO.: 199698822771
Purification and characterization of salivary kallikrein from an insectivore (Scalopus aquaticus): Substrate specificities, immunoreactivity and kinetic analyses.
AUTHOR: Richards G P; Zintz C; Chao J; Chao L(a)
AUTHOR ADDRESS: (a)Med. Univ. South Carolina, Dep. Biochemistry Molecular Biol., 171 Ashley Ave., Charleston, SC 29**USA
JOURNAL: Archives of Biochemistry and Biophysics 329 (1):p104-112 1996 ISSN: 0003-9861

DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: We report the successful one-step separation of tissue kallikrein from the salivary glands of an insectivore, the Eastern Atlantic mole (Scalopus aquaticus) by perfusion chromatography. Purified mole salivary kallikrein was characterized as a 30-kDa serine proteinase with a pI of 5.3 and a pH optimum of 9.0. It was readily recognized by human tissue kallikrein *antibody* in immunoblot analyses. It preferentially hydrolyzes fluorogenic peptidyl substrates with arginyl residues, rather than lysyl residues at the P1 substrate recognition site, indicating that it is like other mammalian kallikreins. Mole kallikrein efficiently releases kinin from low molecular weight human, dog, and bovine kininogen substrates with specific activities similar to that of human tissue kallikrein. Steady state kinetics performed with the synthetic tripeptidyl substrates, Phe-Phe-Arg-, Pro-Phe-Arg-, and Val-Leu-Arg-7-amino-4-methylcoumarin, gave K-m values for mole kallikrein of 3.3, 46.1, and 2.8 mu-M, respectively, and specificity constants, k-cat/K-m, of 3818, 165, and 8714 s-1 *pM*-*1*, respectively. Mole kallikrein, when compared with human and rat tissue kallikreins, more closely resembles human kallikrein based on immunoreactivity and kininogenase activity. Mole kallikrein appears to be a member of a single gene or small multigene family. S. aquaticus is recommended for studying the evolution of mammalian proteins and may offer advantages over rodent models for biomedical research.

1996

6/3,AB/31 (Item 6 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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09917944 BIOSIS NO.: 199598372862
The regulatory site of functional GTP binding protein coupled to the high affinity cholecystokinin receptor and phospholipase A-2 pathway is on the G-beta subunit of G-q protein in pancreatic acini.
AUTHOR: Tsunoda Yasuhiro; Owyang Chung
AUTHOR ADDRESS: Dep. Internal Med., Univ. Mich., Ann Arbor, MI 48109**USA JOURNAL: Biochemical and Biophysical Research Communications 211 (2):p 648-655 1995
ISSN: 0006-291X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A non-hydrolysable guanosine nucleotide analog, GTP(S) at 200 mu-M, stimulated amylase secretion which was inhibited by an anti-phospholipase A-2 (PLA-2) *antibody* in permeabilized pancreatic acini, indicating that the PLA-2 pathway is linked to the GTP binding protein. A high affinity cholecystokinin (CCK) receptor agonist, CCK-OPE (10 mu-M), and a low affinity receptor agonist, CCK-8 (0.1 mu-M), both caused amylase secretion in permeabilized cells. The action of CCK-OPE was abolished by the GB *antibody* but not by the G-alpha-q,11 *antibody*, whereas the opposite was true of the CCK-8 response. Biscoclaurine alkaloid isotetrandrine (10 mu-M), a specific inhibitor of PLA-2-coupled G proteins, abolished Ca-2+ oscillations and amylase secretion induced by CCK-OPE (0.1100 nM), but not by CCK-8 (10 pM) in intact acini. Gp antagonist-2A (10 mu-M), which inhibits the activation of Gq, also inhibited the actions of CCK-OPE (10 *pM*-*1* mu-M) in intact acini. These observations indicate that the functional unit of the heterotrimeric G protein coupled to the high affinity CCK receptor appears to be different from that linked to the low affinity CCK receptor/G-q-alpha pathway. The regulatory site of this G protein coupled to the high affinity CCK receptor is on the beta subunit of G-q protein which elicits Ca-2+ oscillations and monophasic amylase secretion via the PLA-2 pathway.

1995

6/3,AB/32 (Item 7 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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09441127 BIOSIS NO.: 199497449497
Cryptic nature of envelope V3 region epitopes protects primary monocytotropic human immunodeficiency virus type 1 from *antibody* neutralization.
AUTHOR: Bou-Habib Dumith Chequer; Roderiquez Gregory; Oravecz Tamas; Berman Phillip W; Lusso Paolo; Norcross Michael A(a)
AUTHOR ADDRESS: (a)Div. Hematol. Products, Center Biol. Evaluation Res., Food Drug Administration, NIH, Building 29**USA
JOURNAL: Journal of Virology 68 (9):p6006-6013 1994
ISSN: 0022-538X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Characterization of biological and immunological properties of human immunodeficiency virus type 1 (HIV-1) is critical to developing effective therapies and vaccines for AIDS. With the use of a novel CD4+ T-cell line (*PM*-*1*) permissive to infection by

both monocytotropic (MT) and T-cell-tropic virus types, we present a comparative analysis of the immunological properties of a prototypic primary MT isolate of HIV-1 strain JR-CSF (MT-CSF) with those of a T-cell-tropic variant (T-CSF) of the same virus, which emerged spontaneously in vitro. The parental MT-CSF infected only *PM*- *1* cells and was markedly resistant to neutralization by sera from HIV-1-infected individuals, rabbit antiserum to recombinant MT-CSF gp120, and anti-V3 monoclonal antibodies. The T-CSF variant infected a variety of CD4+ T-cell lines, contained positively charged amino acid substitutions in the gp120 V3 region, and was highly sensitive to *antibody* neutralization. Neutralization and *antibody* staining of T-CSF-expressing cells were significantly inhibited by HIV-1 V3 peptides; in contrast, the MT strain showed only weak V3-specific binding of polyclonal and monoclonal antibodies. Exposure of *PM*-*1* cells to a mixture of both viruses in the presence of human anti-HIV-1 neutralizing antiserum resulted in infection with only MT-CSF. These results demonstrate that although the V3 region of MT viruses is immunogenic, the target epitopes in the V3 principal neutralizing domain on the membrane form of the MT envelope appear to be cryptic or hidden from blocking antibodies.

1994

6/3,AB/33 (Item 8 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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09121766 BIOSIS NO.: 199497130136
Direct and correlated responses to multitrait, divergent selection for immunocompetence.
AUTHOR: Kean R P; Cahaner A; Freeman A E; Lamont S J(a)
AUTHOR ADDRESS: (a)Dep. Animal Sci., Iowa State Univ., Ames, IA 50011**USA JOURNAL: Poultry Science 73 (1):p18-32 1994
ISSN: 0032-5791
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Leghorn lines had been selected for an immunocompetence index based on four traits: *antibody* production to Mycoplasma gallisepticum (MG) and Pasteurella multocida (PM) vaccines, reticuloendothelial clearance of colloidal carbon (CCA), and cell-mediated, wing web response to phytohemagglutinin (PHA). The purpose of this study was to produce replicated lines of chickens with divergent levels of multitrait immunocompetence by index selection. The objectives of analyses of Generations 5 to 7 of this study was to characterize these lines with respect to immune-response traits, correlations among these traits, and correlated responses in other important production traits. Differences (P lt .05) existed between the lines selected for high or low immune response and between the two replicates in mean breeding values and in individual immune-response traits. Averages of heritability estimates, weighted by number of offspring and pooled across three generations (two cycles of selection), estimated by using sire variance components and parent-offspring correlations were, respectively, .16 and .09 for the index, .31 and .08 for MG, .21 and -.02 for PM, .06 and .05 for CCA, and .08 and .12 for PHA. Realized heritabilities (response divided by effective selection differential) pooled across the two selection cycles, were .19 and .11 for the index, .06 and -.01 for MG, .44 and.32 for *PM*, *1*.52 and -1.21 for CCA, and .48 and. 15 for PHA, for Replicates 1 and 2, respectively. Phenotypic correlations among traits were generally small, and several estimates were negative. Estimates of genetic correlation varied widely. Juvenile and adult body weights, age of first egg, 32-wk egg weight, and rate of egg production were analyzed to evaluate effects of selection on these traits of direct economic importance. Very few differences were noted.

1994

6/3,AB/34 (Item 9 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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08821437 BIOSIS NO.: 199395110788
Reshaping a human *antibody* to inhibit the interleukin 6-dependent tumor cell growth.
AUTHOR: Sato Koh; Tsuchiya Masayuki(a); Saldanha Jose; Koishihara Yasuo; Ohsugi Yoshiyuki; Kishimoto Tadamitsu; Bendig Mary M
AUTHOR ADDRESS: (a)Fuji-Gotemba Research Lab., Chugai Pharmaceutical Co. Ltd., 1-135 Komakado, Gotemba, Shizuoka 41**Japan
JOURNAL: Cancer Research 53 (4):p851-856 1993
ISSN: 0008-5472
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The mouse *PM*-*1* monoclonal *antibody* binds to the human interleukin 6 receptor, inhibits IL-6 functions, and shows strong antitumor cell activity against multiple myeloma cells. In order to be effective as a therapeutic agent administered to human patients in repeated doses, reshaped human *PM*-*1* antibodies consisting of human REI-based light chain and NEW-based heavy chain variable regions were designed

and constructed with the assistance of a structural model of the mouse *PM*-*1* variable regions. The best reshaped human *PM*-*1* *antibody* is equivalent to mouse or chimeric *PM*-*1* *antibody* in terms of antigen binding and growth inhibition against multiple myeloma cells. Only a few minor changes in the human framework regions were required to recreate the mouse *PM*- *1* antigen-binding site within a human *antibody*. The reshaped human *PM*-*1* *antibody*, therefore, could be efficacious in human multiple myeloma patients.

1993

6/3,AB/35 (Item 10 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

08141538 BIOSIS NO.: 000093128686
TROPHOBLAST-DERIVED TUMOR NECROSIS FACTOR-ALPHA INDUCES RELEASE OF HUMAN CHORIONIC GONADOTROPIN USING INTERLEUKIN-6 IL-6 AND
IL-6-RECEPTOR-DEPENDENT SYSTEM IN THE NORMAL HUMAN TROPHOBLASTS AUTHOR: LI Y; MATSUZAKI N; MASUHIRO K; KAMEDA T; TANIGUCHI T; SAJI F; YONE K; TANIZAWA O AUTHOR ADDRESS: 1-1-50 FUKUSHIMA, FUKUSHIMA-KU, OSAKA 553, JPN. JOURNAL: J CLIN ENDOCRINOL METAB 74 (1). 1992. 184-191. 1992 FULL JOURNAL NAME: Journal of Clinical Endocrinology & Metabolism CODEN: JCEMA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: The titer of tumor necrosis factor-.alpha. (TNF.alpha.) secreted by placental blocks was determined by enzyme immunoassay. The source of placental TNF.alpha. was immunohistochemically demonstrated with monoclonal anti-TNF.alpha. *antibody* to be only trophoblasts. Purified trophoblasts produced 174.4 ng/L TNF.alpha. by 24 h of culture in vitro. To investigate the role of TNF.alpha. in placental hormonogenesis, purified trophoblasts were stimulated with recombinant TNF.alpha. (rTNF.alpha.) to determine the hCG titer by enzyme immunoassay. Trophoblasts stimulated with rTNF.alpha. released hCG in a dose-dependent fashion with kinetics similar to those of recombinant interleukin-1 (rIL-1)-stimulated trophoblasts. The stimulated trophoblasts released IL-6 before hCG, but failed to show hCG release when pretreated with anti-IL-6 receptor (anti-IL-6R) monoclonal *antibody* *PM*-*1*. However, the pretreatment of trophoblasts with *PM*-*1* did not interfere with rTNF-.alpha.-induced IL-6 release, ruling out the possibility of a nonspecific toxic effect of *PM*-*1* on trophoblasts. These results suggest that trophoblast-derived TNF.alpha. induced IL-6 release and then activated the IL-6 -R system in trophoblasts to release hCG. Since IL-1 has also been demonstrated to induce similar release of IL-6 and hCG from trophoblasts, the effects of TNF.alpha. and IL-1 on these trophoblast functions were also examined. Simultaneous stimulation of trophoblasts with rTNF.alpha. and .gamma.IL-1.alpha. resulted in synergistic enhancement of IL-6 release, subsequently leading to enhanced hCG release. Collectivity, trophoblast-derived TNF.alpha. and IL-1 synergistically regulated the level of IL-6 secreted by trophoblasts, the magnitude of which determined the level of hCG released by activating the IL-6-R system in trophoblasts.

1992

6/3,AB/36 (Item 11 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

07379913 BIOSIS NO.: 000091006593
PARADOXICAL ENHANCEMENT OF INTERLEUKIN-2-MEDIATED CYTOTOXICITY AGAINST K562 CELLS BY ADDITION OF A LOW DOSE OF METHOTREXATE
AUTHOR: NAKARAI T; UENO Y; UENO Y; KOIZUMI S AUTHOR ADDRESS: DEP. PEDIATRICS, KANAZAWA UNIV. SCH. MED., 13-1 TAKARA-MACHI, KANAZAWA 920, JAPAN.
JOURNAL: CANCER IMMUNOL IMMUNOTHER 32 (1). 1990. 8-12. 1990 FULL JOURNAL NAME: Cancer Immunology Immunotherapy
CODEN: CIIMD
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: In vitro effects of methotrexate (MTX) on interleukin-2(IL-2)-mediated cytotoxicity of peripheral blood mononculear cells (PBMC) were studied. PBMC were incubated with human recombinant IL-2 (25 U/ml) for 72 h; during the last 24 h, various concentrations (10 *pM*-*1* 1 .mu.M) of MTX were added to the culture. Cytotoxicity against k562 cells was measured by a 4-h 51Cr-release assay. The IL-2-mediated cytotoxicity was paradoxically increased at around a concentration (10 nM) MTX. Such a low concentration of MTX showed no anti-proliferative effect on cell growth. This enhancement with 10 nM MTX was shown only in an E-rosette+ (E+) population, but not in E-rosette- (E-). In addition, when E+ cells were treated with an anti-CD16 monoclonal *antibody* plus complement after incubation with IL-2 and MTX, MTX-induced enhancement was lost,

suggesting that an E+CD16+ cell population was mainly involved in this augmentation. Positively sorted E+CD16+ cells showed similar enhancement of cytotoxicity after treatment with IL-2 plus MTX. On the other hand, MTX treatment did not show the phenotypical changes including of the E+CD16+ cells, indicating that this treatment did not affect the differentiation and proliferation of the specific cell subset. Our results indicate that a low dose of MTX could have a role in the regulation of immunological anti-cancer surveillance systems through the natural killer and lymphokine-activated cytotoxic cells.

1990

6/3,AB/37 (Item 12 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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07318029 BIOSIS NO.: 000090097928
TROPHOBLAST-DERIVED INTERLEUKIN-6 IL-6 REGULATES HUMAN CHORIONIC GONADOTROPIN RELEASE THROUGH IL-6 RECEPTOR ON HUMAN TROPHOBLASTS AUTHOR: NISHINO E; MATSUZAKI N; MASUHIRO K; KAMEDA T; TANIGUCHI T; TAKAGI T ; SAJI F; TANIZAWA O
AUTHOR ADDRESS: DEP. OBSTETRICS GYNECOLOGY, OSAKA UNIVERSITY MEDICAL SCHOOL, 1-1-50 FUKUSHIMA, FUKUSHIMA-KU, OSAKA 553, JPN.
JOURNAL: J CLIN ENDOCRINOL METAB 71 (2). 1990. 436-441. 1990 FULL JOURNAL NAME: Journal of Clinical Endocrinology & Metabolism CODEN: JCEMA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: We examined the capacity of trophoblast-derived interleukin-6 (UL-6) to stimulate secretion of placental hormones, including hCG. IL-6 stimulated hCG secretion by trophoblasts to a level similar to that stimulated by a GnRH analog. The analog, however, released hCG by an IL-6-independent mechanism because *PM*-*1*, a monoclonal *antibody* specific for IL-6 receptors (R), failed to block GnRH-mediated responses, but completely blocked IL-6 mediated hCG secretion, suggesting the existence of two distinct regulatory pathways for hCG release. Immunohistochemical analysis with another IL-6-R-specific *antibody*. MT-18, showed that IL-6-R was located only on the trophoblast layer of the placenta. Our data revealed the existence of a local regulatory network by which trophoblast-derived IL-6 interacts with IL-6-R on the trophoblasts, resulting in hCG release. Thus, two different regulatory networks, an IL-6 and IL-6-R system and a GnRH and GnRH-R system, regulate hCG release by human trophoblasts independently.

1990

6/3,AB/38 (Item 13 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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07280442 BIOSIS NO.: 000090060329
MOLECULAR VARIANTS OF CHOLECYSTOKININ AFTER ENDOGENOUS STIMULATION IN HUMANS A TIME STUDY
AUTHOR: EYSSELEIN V E; EBERLEIN G A; HESSE W H; SCHAEFFER M; GRANDT D; WILLIAMS R; GOEBELL H; REEVE J R JR
AUTHOR ADDRESS: DEP. GASTROENTEROL., HARBOR-UCLA MED. CENT., 1000 W. CARSON, ST., TORRANCE, CALIF. 90509.
JOURNAL: AM J PHYSIOL 258 (6 PART 1). 1990. G951-G957. 1990 FULL JOURNAL NAME: American Journal of Physiology
CODEN: AJPHA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: The time-dependent release of molecular variants of cholecystokinin (CCK) into the circulation was studied before and 1,2, and 4 h after a test meal in six healthy volunteers. At each time period, 100 ml of blood were drawn in a manner to inhibit CCK degradation. Plasma was formed and CCK concentrated by Sep-Pak C18 cartridge chromatography. Molecular variants of CCK and gastrin were well separated from each other by high-performance liquid chromatography (HPLC). Molecular forms of CCK and gastrin were measured by radioimmunoassay using an *antibody* that requires the presence of the carboxyl-terminal phenylalanine amide for full recognition, implying that biologically active forms were detected. HPLC elution positions of gastrin forms were determined using a gastrin-specific *antibody*. Chromatographic separation of CCK from gastrin forms was complete, allowing separate integration of gastrin and CCK forms. Therefore no substraction of gastrin-like immunoreactivity from CCK-like immunoreactivity (CCK-LI) was necessary and CCK-LI could be directly determined. Peaks of CCK-LI were integrated in the column eluates and the plasma concentrations were calculated. Total plasma CCK-LI rose from a value of 2.4 .+-. 0.6 pM before the test meal to 6.4 .+-. 0.8, 6.6 .+-. 0.9, and 5.8 .+-. 1.2 *pM* *1*, 2, and 4 h postprandially. The major molecular forms released into the circulation eluted on HPLC in the position of CCK-58 and CCK-39 (which coelutes with CCK-33). Minor amounts were detected in the position of CCK-8. There was no significant difference in the relative proportions of the

molecular forms released at the different time periods. The high proportions of CCK-58 in human plasma indicate that it expresses a major portion of CCK's biological activity.

1990

6/3,AB/39 (Item 14 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

06563144 BIOSIS NO.: 000087005305
EFFECT OF INTERLEUKIN 1 BETA ON TRANSDUCING MECHANISMS IN 235-1 CLONAL PITUITARY CELLS PART II MODULATION OF CALCIUM FLUXES
AUTHOR: SCHETTINI G; MEUCCI O; FLORIO T; SCALA G; LANDOLFI E; GRIMALDI M AUTHOR ADDRESS: DEP. PHARMACOL., II SCH. MED., VIA PANSINI 5, 80131 NAPOLI, ITALY.
JOURNAL: BIOCHEM BIOPHYS RES COMMUN 155 (3). 1988. 1097-1104. 1988 FULL JOURNAL NAME: Biochemical and Biophysical Research Communications
CODEN: BBRCA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: In the present study we investigated the effect of the interleukin 1 beta on intracellular free calcium concentrations in 235-1 cell line both in basal conditions and after stimulation by the calcium channel activator maitotoxin. Interleukin 1 beta (from 0.01 pM to 10 nM) was unable to significantly affect basal cytosolic free calcium levels in acute conditions. The preincubation of these cells with interleukin 1 beta for 48th modulates maitotoxin stimulation of calcium fluxes without modifying basal intracellular free calcium levels. Low concentrations of interleukin 1 beta (0.01 *pM*, *1* pM) caused a marked reduction of intracellular free calcium concentrations increase induced by maitotoxin while higher doses of the monokine potentiated maitotoxin stimulation of calcium fluxes. The specificity of interleukin 1 beta effect was tested by means of polyclonal anti-interleukin 1 beta *antibody* (titer 1:100) which significantly abolished the inhibitory effect of interleukin 1 beta on free cytosolic calcium levels. These results show that a long lasting interaction of interleukin 1 beta with its receptor is able to influence voltage-sensitive calcium channels activation induced by maitotoxin in 235-1 cells.

1988

6/3,AB/40 (Item 15 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

04682591 BIOSIS NO.: 000079095720
IMMUNOGENETIC STUDIES OF JUVENILE DERMATOMYOSITIS 3. STUDY OF *ANTIBODY* TO ORGAN-SPECIFIC AND NUCLEAR ANTIGENS
AUTHOR: PACHMAN L M; FRIEDMAN J M; MARYJOWSKI-SWEENEY M L; JONNASON O; RADVANY R M; SHARP G C; COBB M A; BATTLES N D; CROWE W E; ET AL AUTHOR ADDRESS: DIV. IMMUNOLOGY/RHEUMATOLOGY, CHILDREN'S MEMORIAL HOSPITAL, 2300 CHILDREN'S PLAZA, CHICAGO, IL 60614.
JOURNAL: ARTHRITIS RHEUM 28 (2). 1985. 151-157. 1985
FULL JOURNAL NAME: Arthritis and Rheumatism
CODEN: ARHEA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Children (90) with definite juvenile dermatomyositis (JDMS), who had been HLA typed, were tested for the presence of tissue or organ-specific antibodies. Sixty had active disease at the time of study. The mean disease duration was 4 yr and 30 had soft tissue calcifications. The following autoantibodies were sought: thyroid, gastric parietal cells, smooth muscle, striated muscle, microsomes, mitochondria, DNA, extractable nuclear antigen, Sm, *PM*-*1*, antinuclear *antibody* (ANA) and rheumatoid factor. Only the ANA and *PM*- *1* were more frequent in patients than in controls ($P < 0.0002$ and $P < 0.001$, respectively). Higher levels of immune complexes ($P < 0.01$) were in sera from patients with JDMS than in sera from controls and were correlated with the presence of ANA in patients ($P < 0.01$). Soft tissue calcification was not associated with any autoantibody or HLA antigen, but with disease duration and activity ($P < 0.001$ and $P < 0.05$, respectively). There was no association between the occurrence of any autoantibody and the presence of HLA-B8 or DR3 among the white patients with JDMS. The frequency of autoantibodies in 43 full siblings of children with JDMS was not increased. Children with JDMS, with or without HLA-B8/DR3, do not show evidence of a generalized nonspecific *antibody* response to tissue antigens. The significance of the increased *antibody* to nuclear antigens ANA and *PM*-*1* remains to be determined.

1985

6/3,AB/41 (Item 16 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

04627749 BIOSIS NO.: 000079040786
CLINICAL RELEVANCE OF *PM*-*1* *ANTIBODY* AND PHYSICOCHEMICAL CHARACTERIZATION OF *PM*-*1* ANTIGEN
AUTHOR: TREADWELL E L; ALSPAUGH M A; WOLFE J F; SHARP G C
AUTHOR ADDRESS: N-403 UNIV. MO. HEALTH SCI. CENT., COLUMBIA, S.C. 65212. JOURNAL: J RHEUMATOL 11 (5). 1984. 658-662. 1984
FULL JOURNAL NAME: Journal of Rheumatology
CODEN: JRHUA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Using an improved immunodiffusion test with partially purified antigen, *PM*-*1* *antibody* was identified in the serum of 18 patients. In 67% this system was associated with a polymyositis-scleroderma overlap, it occurred less frequently in polymyositis, dermatomyositis and scleroderma, and was not detected in other rheumatic diseases. The predominant clinical features of *PM*- *1* positive patients were muscle weakness, sclerodactyly. Raynaud's phenomenon and pulmonary disease, widespread sclerodermatous features were infrequent. Characterization of the *PM*-*1* antigen showed it to be a heat sensitive, trypsin sensitive acidic protein associated with the cell nucleus and possibly with nucleoli.

1984

6/3,AB/42 (Item 17 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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04338205 BIOSIS NO.: 000078067749
STANDARDIZATION OF THE IMMUNO FLUORESCENCE TEST FOR AUTO *ANTIBODY* TO NUCLEAR ANTIGENS USE OF REFERENCE SERA OF DEFINED *ANTIBODY* SPECIFICITY
AUTHOR: MOLDEN D P; NAKAMURA R M; TAN E M
AUTHOR ADDRESS: DEP. PATHOL., SCRIPPS CLINIC RES. FOUNDATION, 10666 N. TORREY PINES ROAD, LA JOLLA, CALIF. 92037.
JOURNAL: AM J CLIN PATHOL 82 (1). 1984. 57-66. 1984
FULL JOURNAL NAME: American Journal of Clinical Pathology
CODEN: AJCPA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Standardization of the indirect immunofluorescence antinuclear *antibody* (IF-ANA) test can be improved for a given substrate with use of reference [human] ANA sera, uniform assay conditions, and standardization of optical systems. To accomplish this, reference sera from the Arthritis Foundation with defined *antibody* specificities for nDNA [nuclear DNA], SS-B, RNP [ribonucleoprotein] Sm, nucleoli and speckled pattern were reacted with commonly used IF-ANA substrates, mouse kidney sections, KB [human epidermoid carcinoma] and HEPL-2 [human laryngeal carcinoma] tissue culture cells. Reagents and assay conditions used were those provided with the substrates in commercially available IF-ANA kits. A microscope slide with graded intensities of fluorescent beads was used to standardize microscope flourescence intensity readings. The flourescence pattern and intensity results should be directly comparable to results obtained in other laboratories for the 6 antibodies for which reference sera are available. Although no defined sera are widely available for SS-A, Scl-70, *PM*-*1* and centromere antibodies, the ability of each substrate to detect these ANA as well as mitochondrial, smooth muscle, ribosomal and microsomal antibodies also was tested. HEp-2 cells and KB cells were superior to mouse kidney sections for detection of SS-A, Scl-70, *PM*-*1* and centromere ANA. Mouse kidney sections were superior for screening of sera for the absence of ANA and for detection of smooth muscle and liver-kidney microsomal antibodies. Other antibodies were detected with equal sensitivity with all substrates and each of the 3 ANA kits used in the study performed satisfactorily. Use of reference sera and optical standardization are recommended for IF-ANA testing.

1984

6/3,AB/43 (Item 18 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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03543780 BIOSIS NO.: 000073046861
MULTIPLE ANTI NUCLEAR ANTIBODIES IN MIXED CONNECTIVE TISSUE DISEASE A PATIENT WITH AN UNUSUAL *ANTIBODY* PROFILE
AUTHOR: HEPBURN B
AUTHOR ADDRESS: RARITAN VALLEY HOSPITAL, GREEN BROOK, NJ 08812. JOURNAL: J RHEUMATOL 8 (4). 1981. 635-638. 1981
FULL JOURNAL NAME: Journal of Rheumatology
CODEN: JRHUA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: A patient with antibodies to RNP [ribonucleoprotein], n[native]-DNA, Sm antigen and *PM*-*1* is described. The 1st clinical manifestations of

connective tissue disease appeared at age 10 yr. A cystic pulmonary lesion, pericardial effusion and nephropathy appeared after 7 yr of disease limited to skin and joints. Pulmonary, pericardial and renal disease all appeared to respond to corticosteroid therapy.

1981

6/3,AB/44 (Item 19 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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03458714 BIOSIS NO.: 000023031802
THE *PM*-*1* *ANTIBODY* TEST IN A PATIENT WITH RHEUMATIC COMPLAINTS
AUTHOR: SOLOMON S D
AUTHOR ADDRESS: 1860 GREENTREE RD., CHERRY HILL, N.J. 08003. JOURNAL: J RHEUMATOL 9 (1). 1982. 157-159. 1982
FULL JOURNAL NAME: Journal of Rheumatology
CODEN: JRHUA
RECORD TYPE: Citation
LANGUAGE: ENGLISH
1982

6/3,AB/45 (Item 20 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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02970329 BIOSIS NO.: 000069078447
JUVENILE DERMATO MYOSITIS A CLINICAL AND IMMUNOLOGIC STUDY AUTHOR: PACHMAN L M; COOKE N
AUTHOR ADDRESS: CHILD. MEML. HOSP., 2300 CHILDREN'S PL., CHICAGO, ILL. 60614, USA.
JOURNAL: J PEDIATR 96 (2). 1980. 226-234. 1980
FULL JOURNAL NAME: Journal of Pediatrics
CODEN: JOPDA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Children (21) were diagnosed as having juvenile dermatomyositis on the basis of the strict criteria of Bohan and Peter. In addition to typical skin and muscle changes, abnormalities of esophageal motility (8 of 19), pulmonary function (14 of 17), ECG (10 of 20) and gastrointestinal absorption of D-xylose (2 of 8) with active disease were observed. Clinical signs of other collagen vascular disease appeared in 5 children. Serologic evaluation demonstrated that ANA [antinuclear *antibody*] and rheumatoid factor were transiently positive in 6; 1 child developed a persistently positive rheumatoid factor after 4 years of disease inactivity. *Antibody* to ENA was negative in all, but *antibody* to *PM*-*1* [polymyositis] antigen was present in 4 of 18. Six had a low C3 [complement component 3] or C4; evidence of immune complexes was demonstrated by Clq or Raji [Burkitt's lymphoma] binding in 8 with active disease. One child was Ig[immunoglobulin]A deficient. The HLA-B8 antigen was present in 72% of the Caucasian children as compared with the expected incidence of 21%. Classical dermatomyositis in children has more systemic involvement then previously appreciated, may be related to the presence of circulating immune complexes and appears to be under immunogenetic control.
1980

6/3,AB/46 (Item 21 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
(c) 2003 BIOSIS. All rts. reserv.

02562995 BIOSIS NO.: 000017011052
DISEASE PATTERN OF PATIENTS WITH *PM*-*1* *ANTIBODY* AUTHOR: WOLFE J F; ADELSTEIN E; SHARP G C
JOURNAL: CLIN RES 26 (3). 1978 387A 1978
FULL JOURNAL NAME: Clinical Research
CODEN: CLREA
DOCUMENT TYPE: Meeting
RECORD TYPE: Citation
1978

6/3,AB/47 (Item 22 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
(c) 2003 BIOSIS. All rts. reserv.

02136475 BIOSIS NO.: 000063051475
ANTI NUCLEAR *ANTIBODY* WITH DISTINCT SPECIFICITY FOR POLY MYOSITIS AUTHOR: WOLFE J F; ADELSTEIN E; SHARP G C
JOURNAL: J CLIN INVEST 59 (1). 1977 176-178. 1977
FULL JOURNAL NAME: Journal of Clinical Investigation
CODEN: JCINA
RECORD TYPE: Abstract

ABSTRACT: In the course of studying antinuclear antibodies in the rheumatic diseases, a new precipitin reaction (provisionally referred to as *PM*-*1*) was observed between calf thymus nuclear extract and polymyositis sera. The objectives of this study were to further define the immunologic nature of this reaction and to determine its specificity for polymyositis. Immunodiffusion studies using calf thymus nuclear extract revealed the *PM*-*1* precipitin line in 17 of 28 patients with polymyositis. This reaction was not produced by sera of 460 patients with other diseases. Enzyme and heat treatments of the nuclear extract showed that *PM*-*1* was distinct from native DNA,

ribonucleoprotein and Sm antigens. Fractionation of *PM*-*1* -positive serum by 30% ammonium sulfate and Sephadex G-200 chromatography revealed that the factor producing the *PM*-*1* precipitin reaction was in a serum fraction which showed only IgG [immunoglobulin G] by immunoelectrophoresis against anti-whole human serum. Because of the apparent strong specificity, the *PM*-*1* system may represent a marker *antibody* for polymyositis.

1977
? ds

Set Items Description
S1 556 PM()1 OR MR16()1
S2 545 PM()1
S3 11 MR16()1
S4 54 S2 AND ANTIBODY
S5 7 S4 AND PY>1999
S6 47 S4 NOT S5
? s s3 and antibody
11 S3
748137 ANTIBODY
S7 11 S3 AND ANTIBODY
? t s7/3,ab/all

7/3,AB/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.

14812911 22301843 PMID: 12413742
Characterization of anti-mouse interleukin-6 receptor *antibody*. Okazaki Makoto; Yamada Yoshiki; Nishimoto Norihiro; Yoshizaki Kazuyuki; Mihara Masahiko
Fuji-Gotemba Research Laboratories, Chugai Pharmaceutical Co. Ltd, 135 Komakado 1-chome, Gotemba-shi, Shizuoka 412-8513, Japan.
Immunology letters (Netherlands) Dec 3 2002, 84 (3) p231-40, ISSN 0165-2478 Journal Code: 7910006
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Hybridoma that produces rat anti-mouse interleukin 6 receptor (IL-6R) *antibody*, *MR16*-*1*, was established by the fusion of mouse P3U1 myeloma cells and spleen cells from mouse soluble IL-6R (sIL-6R)-immunized Wistar rat. In the present study, we examined the characteristics of *MR16*-*1* in vitro and in vivo. *MR16*- *1* bound to mouse sIL-6R dose-dependently. *MR16*-*1* suppressed IL-6-induced proliferation of 7TD1 cells in a dose-dependent manner and this inhibitory effect was reversed by the addition of a higher concentration of IL-6. Cross-reactivity study using T cells from mouse, rat, and human revealed that *MR16*-*1* did not cross-react with human and rat IL-6R. Binding region analysis using several human-mouse chimeric IL-6Rs showed that half of the fibronectin domain II of mouse IL-6R (amino acids 214-285) was required for *MR16*-*1* binding. Furthermore, *MR16*-*1* completely suppressed IL-6-induced *antibody* production in DNP-KLH immunized mice. These lines of evidence demonstrate that *MR16*-*1* is useful to investigate the physiological and pathological roles of IL-6 and sIL-6R in mice.

7/3,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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14812910 22301842 PMID: 12413741
Influences of anti-mouse interleukin-6 receptor *antibody* on immune responses in mice.
Mihara Masahiko; Nishimoto Norihiro; Yoshizaki Kazuyuki; Suzuki Takashi Fuji-Gotemba Research Laboratories, Chugai Pharmaceutical Co. Ltd., 135, Komakado 1-chome, Gotemba-shi, Shizuoka 412-8513, Japan. miharamsh@chugai-pharm.co.jp
Immunology letters (Netherlands) Dec 3 2002, 84 (3) p223-9, ISSN 0165-2478 Journal Code: 7910006
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
In the present study, we examined the effect of anti-IL-6 receptor *antibody* (*MR16*-*1*) on humoral and cellular immune responses in mice. *MR16*-*1* did not affect antigen-specific *antibody* production in either the primary or secondary response in mice immunized with dinitro-phenyl (DNP)-keyhole limpet haemocyanin (KLH) in saline. DNP-KLH immunization with complete Freund's adjuvant (CFA) markedly augmented anti-DNP *antibody* production and induced interleukin 6 (IL-6) production in serum. In this case, *MR16*-*1* significantly suppressed *antibody* production and further increased serum IL-6 levels. Regarding the cellular response, we studied the effect on the delayed-type hypersensitivity (DTH) response. DTH response was induced in mice by the immunization with Mycobacterium butyricum with incomplete Freund's adjuvant and following antigen challenge into the footpad 14 days after immunization. When *MR16*-*1* was injected immediately after immunization, the DTH response was significantly suppressed and enlargement of the spleen was also suppressed. This suppressive effect was observed, when *MR16*-*1* was administered on day 0, but not on days 5 and 10. Again, serum IL-6 levels were much higher in *MR16*-*1* -treated mice compared with controls. Furthermore, spleen cells from control mice released IL-2 and INFgamma by the stimulation of antigen in vitro. In contrast, spleen cells from *MR16*-*1*

-treated mice produced these cytokines at a marginal level. In contrast, *MR16*-*1* did not suppress the DTH response, when it was injected immediately after antigen challenge. Our results suggest that IL-6 does not always involve *antibody* production, although IL-6 augments *antibody* production, and that IL-6 is essential for the induction of Th1 cells.

7/3,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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14561403 22520634 PMID: 12633573
Anti-interleukin 6 (IL-6) receptor *antibody* suppresses castleman's disease like symptoms emerged in IL-6 transgenic mice.
Katsume Asao; Saito Hiroyuki; Yamada Yoshiki; Yorozu Keigo; Ueda Otoya; Akamatsu Ken Ichi; Nishimoto Norihiro; Kishimoto Tadamitsu; Yoshizaki Kazuyuki; Ohsugi Yoshiyuki
Department of Medical Science I, School of Health and Sport Sciences, Osaka University, Osaka, Japan
Cytokine (United States) Dec 21 2002, 20 (6) p304-11, ISSN 1043-4666 Journal Code: 9005353
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: In Process
Transgenic mice carrying human IL-6 cDNA fused with a murine major histocompatibility class-I promoter (H-2L(d)) were serially administered with anti-interleukin-6 receptor (IL-6R) monoclonal *antibody* (mAb), *MR16*-*1* , from the age of 4 weeks to estimate its efficacy on a variety of disorders developed in these mice, most of which are similar to the disorders associated with Castleman's disease. In the control mice treated with isotype-matched mAb, a massive and multiple IgG1 plasmacytosis, mesangial proliferative glomerulonephritis, leukocytosis, thrombocytosis, anemia and abnormalities of blood chemical parameters have developed in accordance with the elevation of serum IL-6, and 50% of mice have died of renal failure by 18 weeks of age. In contrast, the treatment with *MR16*-*1* prevented all these symptoms and prolonged the lifetime of the majority of the mice.Thus, the constitutive overexpression of IL-6 caused various disorders, and the treatment with anti-IL-6R mAb completely prevented from these symptoms. These results clearly confirm that IL-6 indeed plays an essential role in the pathogenesis of a variety of disorders. Furthermore, anti-IL-6R mAb could provide novel therapy for Castleman's disease and *MR16*-*1* should be a useful tool to estimate therapeutic potential of IL-6 antagonists in a variety of murine models for human disease.

7/3,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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11652167 99086415 PMID: 9870868
Blockage of interleukin-6 receptor ameliorates joint disease in murine collagen-induced arthritis.
Takagi N; Mihara M; Moriya Y; Nishimoto N; Yoshizaki K; Kishimoto T; Takeda Y; Ohsugi Y
Chugai Pharmaceutical Company Ltd., Shizuoka, Japan.
Arthritis and rheumatism (UNITED STATES) Dec 1998, 41 (12) p2117-21, ISSN 0004-3591 Journal Code: 0370605
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
OBJECTIVE: To clarify the role of interleukin-6 (IL-6) in the pathogenesis of collagen-induced arthritis (CIA). METHODS: CIA was induced by immunizing twice at a 3-week interval with bovine type II collagen (CII) emulsified with complete adjuvant. Rat anti-mouse IL-6 receptor (anti-IL-6R) monoclonal *antibody* *MR16*-*1* or isotype-matched control *antibody* KH-5 was then injected once intraperitoneally. Symptoms of arthritis were evaluated with a visual scoring system, and serum anti-CII *antibody* and IL-6 levels were measured by enzyme-linked immunosorbent assay. In addition, the CII responsiveness of splenic lymphocytes from mice with CIA was examined. RESULTS: In mice with CIA, excess production of IL-6 in sera was observed within 24 hours after the first CII immunization, and then rapidly decreased. Serum IL-6 increased again beginning 14 days after immunization, in conjunction with the onset of arthritis. When *MR16*-*1* was injected immediately after immunization with CII, it inhibited the development of arthritis in a dose-dependent manner. Furthermore, *MR16*-*1* -treated mice exhibited lower serum levels of IgG anti-CII *antibody* and reduced responsiveness of lymphocytes to CII. This suppressive effect was observed when *MR16*-*1* was injected on day 0 or 3, but not when injected on day 7 or 14. CONCLUSION: IL-6 produced after CII immunization appears to play an essential role in the immunity to CII, and anti-IL-6R *antibody* reduces the development of CIA by suppressing IL-6 signal transduction.

7/3,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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11429024 98311541 PMID: 9649207

IL-6 receptor blockage inhibits the onset of autoimmune kidney disease in NZB/W F1 mice.
Mihara M; Takagi N; Takeda Y; Ohsugi Y
Fuji-Gotemba Research Laboratories, Chugai Pharmaceutical Co. Ltd, Shizuoka, Japan.
Clinical and experimental immunology (ENGLAND) Jun 1998, 112 (3) p397-402, ISSN 0009-9104 Journal Code: 0057202
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
In the present study, we examined the preventive effect of anti-mouse IL-6 receptor (IL-6R) *antibody*, *MR16*-*1* , on the development of autoimmune kidney disease in female NZB/W F1 (BWF1) mice. Immunological tolerance to *MR16*-*1* or isotype-matched control *antibody* , KH-5, was induced by the simultaneous administration of anti-CD4 MoAb in mice. Thereafter, mice were intraperitoneally given 0.5 mg of *MR16*-*1* , 0.5 mg of KH-5 or saline once a week from 13 to 64 weeks of age. *MR16*-*1* treatment dramatically suppressed proteinuria and prolonged the survival time of BWF1 mice. Only one out of 10 mice died with high levels of proteinuria throughout the experiment. *MR16*-*1* almost completely suppressed the production of IgG forms of anti-DNA and anti-TNP antibodies, but not the IgM forms of these antibodies. In particular, all IgG subclasses (IgG1, IgG2a, IgG2b and IgG3) of anti-DNA *antibody* production were significantly suppressed. Moreover, serum IgG1, IgG2a and IgG3 levels in *MR16*-*1*-treated mice were lower than those in saline- and KH-5-treated mice, whereas serum IgM and IgA levels were not influenced. In conclusion, *MR16*-*1* potently suppressed the development of autoimmune disease in BWF1 mice, and this was attributed to its effect of specific suppression of IgG class *antibody* production.

7/3,AB/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08024016 94089743 PMID: 8265649
Soluble interleukin-6 receptor triggers osteoclast formation by interleukin 6.
Tamura T; Udagawa N; Takahashi N; Miyaura C; Tanaka S; Yamada Y; Koishihara Y; Ohsugi Y; Kumaki K; Taga T; et al
Department of Biochemistry, School of Dentistry, Showa University, Tokyo, Japan.
Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Dec 15 1993, 90 (24) p11924-8, ISSN 0027-8424 Journal Code: 7505876
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
It has been reported that soluble interleukin (IL)-6 receptor (sIL-6R) is detected in the serum of healthy individuals and its level is increased in patients with multiple myeloma and human immunodeficiency virus infection. Although several reports have suggested that sIL-6R potentiates IL-6 action, its physiological role remains unclear. In this study, we examined the role of sIL-6R on osteoclast formation by IL-6, using a coculture of mouse osteoblasts and bone marrow cells. Neither recombinant mouse IL-6 (mIL-6) nor mouse sIL-6R (smIL-6R) induced osteoclast-like multinucleated cell (MNC) formation when they were added separately. In contrast, simultaneous treatment with mIL-6 and smIL-6R strikingly induced MNC formation. These MNCs satisfied major criteria of authentic osteoclasts, such as tartrate-resistant acid phosphatase (TRAP) activity, calcitonin receptors, and pit formation on dentine slices. The MNC formation induced by mIL-6 and smIL-6R was dose-dependently inhibited by adding monoclonal anti-mouse IL-6R *antibody* (*MR16*-*1*). It is likely that osteoblasts and osteoclast progenitors are capable of transducing a signal from a complex of IL-6 and sIL-6R through gp130, even though they may have no or a very small number of IL-6Rs. Factors such as IL-11, oncostatin M, and leukemia inhibitory factor, which are known to exert their functions through gp130 (the signal-transducing chain of IL-6R), also induced MNC formation in our coculture system. These results suggest that increased circulating or locally produced sIL-6R induces osteoclast formation in the presence of IL-6 mediated by a mechanism involving gp130. This may play an important physiological or pathological role in conditions associated with increased osteoclastic bone resorption.

7/3,AB/7 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

14211368 BIOSIS NO.: 200300205397
Anti-interleukin 6 (IL-6) receptor *antibody* suppresses Castleman's disease like symptoms emerged in IL-6 transgenic mice.
AUTHOR: Katsume Asao(a); Saito Hiroyuki; Yamada Yoshiki; Yorozu Keigo; Ueda Otoya; Akamatsu Ken-ichi; Nishimoto Norihiro; Kishimoto Tadamitsu; Yoshizaki Kazuyuki; Ohsugi Yoshiyuki
AUTHOR ADDRESS: (a)Fuji-Gotemba Research Laboratories, Chugai Pharmaceutical Co. Ltd., 1-135 Komakado, Gotemba-shi, Shizuoka, 412-8513, Japan**Japan E-Mail: katsumeaso@chugai-pharm.co.jp

JOURNAL: Cytokine 20 (6):p304-311 21 December 2002 2002
MEDIUM: print
ISSN: 1043-4666
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Transgenic mice carrying human IL-6 cDNA fused with a murine major histocompatibility class-I promoter (H-2Ld) were serially administered with anti-interleukin-6 receptor (IL-6R) monoclonal *antibody* (mAb), *MR16*-*1*, from the age of 4 weeks to estimate its efficacy on a variety of disorders developed in these mice, most of which are similar to the disorders associated with Castleman's disease. In the control mice treated with isotype-matched mAb, a massive and multiple IgG1 plasmacytosis, mesangial proliferative glomerulonephritis, leukocytosis, thrombocytosis, anemia and abnormalities of blood chemical parameters have developed in accordance with the elevation of serum IL-6, and 50% of mice have died of renal failure by 18 weeks of age. In contrast, the treatment with *MR16*- *1* prevented all these symptoms and prolonged the lifetime of the majority of the mice. Thus, the constitutive overexpression of IL-6 caused various disorders, and the treatment with anti-IL-6R mAb completely prevented from these symptoms. These results clearly confirm that IL-6 indeed plays an essential role in the pathogenesis of a variety of disorders. Furthermore, anti-IL-6R mAb could provide novel therapy for Castleman's disease and *MR16*-*1* should be a useful tool to estimate therapeutic potential of IL-6 antagonists in a variety of murine models for human disease.

2002

7/3,AB/8 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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14033653 BIOSIS NO.: 200300027682
Characterization of anti-mouse interleukin-6 receptor *antibody*. AUTHOR: Okazaki Makoto; Yamada Yoshiki; Nishimoto Norihiro; Yoshizaki Kazuyuki; Mihara Masahiko(a)
AUTHOR ADDRESS: (a)Fuji-Gotemba Research Laboratories, Chugai Pharmaceutical Co. Ltd, 135 Komakado 1-chome, Gotemba-shi, Shizuoka, 412-8513, Japan**Japan E-Mail: miharamsh@chugai-pharm.co.jp
JOURNAL: Immunology Letters 84 (3):p231-240 December 3 2002 2002 MEDIUM: print
ISSN: 0165-2478
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Hybridoma that produces rat anti-mouse interleukin 6 receptor (IL-6R) *antibody*, *MR16*-*1*, was established by the fusion of mouse P3U1 myeloma cells and spleen cells from mouse soluble IL-6R (sIL-6R)-immunized Wistar rat. In the present study, we examined the characteristics of *MR16*-*1* in vitro and in vivo. *MR16*- *1* bound to mouse sIL-6R dose-dependently. *MR16*-*1* suppressed IL-6-induced proliferation of 7TD1 cells in a dose-dependent manner and this inhibitory effect was reversed by the addition of a higher concentration of IL-6. Cross-reactivity study using T cells from mouse, rat, and human revealed that *MR16*-*1* did not cross-react with human and rat IL-6R. Binding region analysis using several human-mouse chimeric IL-6Rs showed that half of the fibronectin domain II of mouse IL-6R (amino acids 214-285) was required for *MR16*-*1* binding. Furthermore, *MR16*-*1* completely suppressed IL-6-induced *antibody* production in DNP-KLH immunized mice. These lines of evidence demonstrate that *MR16*-*1* is useful to investigate the physiological and pathological roles of IL-6 and sIL-6R in mice.

2002

7/3,AB/9 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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14033652 BIOSIS NO.: 200300027681
Influences of anti-mouse interleukin-6 receptor *antibody* on immune responses in mice.
AUTHOR: Mihara Masahiko(a); Nishimoto Norihiro; Yoshizaki Kazuyuki; Suzuki Takashi
AUTHOR ADDRESS: (a)Fuji-Gotemba Research Laboratories, Chugai Pharmaceutical Co. Ltd., 135, Komakado 1-chome, Gotemba-shi, Shizuoka, 412-8513, Japan**Japan E-Mail: miharamsh@chugai-pharm.co.jp
JOURNAL: Immunology Letters 84 (3):p223-229 December 3 2002 2002 MEDIUM: print
ISSN: 0165-2478
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: In the present study, we examined the effect of anti-IL-6 receptor *antibody* (*MR16*-*1*) on humoral and cellular immune responses in mice. *MR16*-*1* did not affect antigen-specific *antibody* production in either the primary or secondary response in mice immunized with dinitro-phenyl (DNP)-keyhole limpet haemocyanin (KLH) in saline. DNP-KLH immunization

with complete Freund's adjuvant (CFA) markedly augmented anti-DNP *antibody* production and induced interleukin 6 (IL-6) production in serum. In this case, *MR16*-*1* significantly suppressed *antibody* production and further increased serum IL-6 levels. Regarding the cellular response, we studied the effect on the delayed-type hypersensitivity (DTH) response. DTH response was induced in mice by the immunization with Mycobacterium butyricum with incomplete Freund's adjuvant and following antigen challenge into the footpad 14 days after immunization. When *MR16*-*1* was injected immediately after immunization, the DTH response was significantly suppressed and enlargement of the spleen was also suppressed. This suppressive effect was observed, when *MR16*-*1* was administered on day 0, but not on days 5 and 10. Again, serum IL-6 levels were much higher in *MR16* -*1*-treated mice compared with controls. Furthermore, spleen cells from control mice released IL-2 and INFgamma by the stimulation of antigen in vitro. In contrast, spleen cells from *MR16*-*1* -treated mice produced these cytokines at a marginal level. In contrast, *MR16*-*1* did not suppress the DTH response, when it was injected immediately after antigen challenge. Our results suggest that IL-6 does not always involve *antibody* production, although IL-6 augments *antibody* production, and that IL-6 is essential for the induction of Th1 cells.

2002

7/3,AB/10 (Item 4 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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11833219 BIOSIS NO.: 199900079328
Blockage of interleukin-6 receptor ameliorates joint disease in murine collagen-induced arthritis.
AUTHOR: Takagi Nobuhiro; Mihara Masahiko; Moriya Yoichiro; Nishimoto Norihiro; Yoshizaki Kazuyuki; Kishimoto Tadamitsu; Takeda Yasuhisa; Ohsugi Yoshiyuki
AUTHOR ADDRESS: Fuji-Gotemba Res. Lab., Chugai Pharmaceutical Co. Ltd., 135 Komakado 1-chome, Gotemba-shi, Shizuoka **Japan
JOURNAL: Arthritis & Rheumatism 41 (12):p2117-2121 Dec., 1998 ISSN: 0004-3591
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Objective. To clarify the role of interleukin-6 (IL-6) in the pathogenesis of collagen-induced arthritis (CIA). Methods. CIA was induced by immunizing twice at a 3-week interval with bovine type II collagen (CII) emulsified with complete adjuvant. Rat anti-mouse IL-6 receptor (anti-IL-6R) monoclonal *antibody* *MR16*-*1* or isotype-matched control *antibody* KH-5 was then injected once intraperitoneally. Symptoms of arthritis were evaluated with a visual scoring system, and serum anti-CII *antibody* and IL-6 levels were measured by enzyme-linked immunosorbent assay. In addition, the CII responsiveness of splenic lymphocytes from mice with CIA was examined. Results. In mice with CIA, excess production of IL-6 in sera was observed within 24 hours after the first CII immunization, and then rapidly decreased. Serum IL-6 increased again beginning 14 days after immunization, in conjunction with the onset of arthritis. When *MR16* -*1* was injected immediately after immunization with CII, it inhibited the development of arthritis in a dose-dependent manner. Furthermore, *MR16*-*1*-treated mice exhibited lower serum levels of IgG anti-CII *antibody* and reduced responsiveness of lymphocytes to CII. This suppressive effect was observed when *MR16*-*1* was injected on day 0 or 3, but not when injected on day 7 or 14. Conclusion. IL-6 produced after CII immunization appears to play an essential role in the immunity to CII, and anti-IL-6R *antibody* reduces the development of CIA by suppressing IL-6 signal transduction.
1998

7/3,AB/11 (Item 5 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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11538401 BIOSIS NO.: 199800319733
IL-6 receptor blockage inhibits the onset of autoimmune kidney disease in NZB/W F1 mice.
AUTHOR: Mihara M(a); Takagi N; Takeda Y; Ohsugi Y
AUTHOR ADDRESS: (a)Fuji-Gotemba Res. Lab., Chugai Pharmaceutical Co. Ltd., 135 Komakado 1-chome, Gotemba-shi, Shizu**Japan
JOURNAL: Clinical and Experimental Immunology 112 (3):p397-402 June, 1998 ISSN: 0009-9104
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: In the present study, we examined the preventive effect of anti-mouse IL-6 receptor (IL-6R) *antibody*, *MR16*-*1*, on the development of autoimmune kidney disease in female NZB/W F1 (BWF1) mice. Immunological tolerance to *MR16*-*1* or isotype-matched control *antibody*, KH-5, was induced by the simultaneous administration of anti-CD4 MoAb in mice. Thereafter, mice were intraperitoneally given 0.5 mg of *MR16*-*1*, 0.5 mg of KH-5 or saline once a week from 13 to 64 weeks of age. *MR16*-*1* treatment dramatically suppressed proteinuria and prolonged the

survival time of BWF, mice. Only one out of 10 mice died with high levels of proteinuria throughout the experiment. *MR16*-*1* almost completely suppressed the production of IgG forms of anti-DNA and anti-TNP antibodies, but not the IgM forms of these antibodies. In particular, all IgG subclasses (IgG1, IgG2a, IgG2b and IgG3) of anti-DNA *antibody* production were significantly suppressed. Moreover, serum IgG1, IgG2a and IgG3 levels in *MR16*-*1*-treated mice were lower than those in saline- and KH-5-treated mice, whereas serum IgM and IgA levels were not influenced. In conclusion, *MR16*-*1* potently suppressed the development of autoimmune disease in BWF1 mice, and this was attributed to its effect of specific suppression of IgG class *antibody* production.

1998
? s il()6 and (pancreatitis or pancreatic()?edema)
>>>File 155 processing for ?EDEMA stopped at ALLERGENY
<------User Break------>
u!
? s il()6 and (pancreatitis or pancreatic()(edema or oedema) >>>Unmatched parentheses
? s il()6 and (pancreatitis or pancreatic()(edema or oedema))
219215 IL
2881919 6
46293 IL(W)6
53800 PANCREATITIS
185720 PANCREATIC
119679 EDEMA
17773 OEDEMA
321 PANCREATIC(W)(EDEMA OR OEDEMA)
S8 284 IL()6 AND (PANCREATITIS OR PANCREATIC()(EDEMA OR OEDEMA))
? s s8 and py>1998
284 S8
4656389 PY>1998
S9 180 S8 AND PY>1998
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284 S8
180 S9
S10 104 S8 NOT S9
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...examined 50 records (50)
...examined 50 records (100)
...completed examining records
S11 78 RD (unique items)
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11/3,AB/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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11679705 99114997 PMID: 9918438
Blood concentrations of polymorphonuclear leucocyte elastase and interleukin-6 are indicators for the occurrence of multiple organ failures at the early stage of acute *pancreatitis*.
Ikei S; Ogawa M; Yamaguchi Y
Department of Surgery II, Kumamoto University Medical School, and Kumamoto National Hospital, Japan.
Journal of gastroenterology and hepatology (AUSTRALIA) Dec 1998, 13 (12) p1274-83, ISSN 0815-9319 Journal Code: 8607909
Comment in J Gastroenterol Hepatol. 1998 Dec;13(12) 1177-9; Comment in PMID 9918421
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We studied potential indicators of severe acute *pancreatitis* by measuring the blood concentrations of various cytokines, polymorphonuclear leucocyte elastase (PMN-E), acute phase reactants, pancreatic amylase (P-AMY), pancreatic elastase-1 (E-1) and white blood cell (WBC) counts in patients with acute *pancreatitis* . In addition, the presence of multiple organ damage was assessed. Subjects consisted of 22 patients with acute *pancreatitis* including severe (n = 11), moderate (n = 4) and mild (n = 7) cases. A significant positive correlation was observed between the number of organs damaged and the peak concentrations of interleukin (*IL*)-*6*, PMN-E, C-reactive protein (CRP) and pancreatic secretory trypsin inhibitor (PSTI). Among these markers, blood concentrations of PMN-E and *IL*-*6* rapidly increased and peaked at the early phase of acute *pancreatitis* whereas CRP and PSTI did not. The elevation of PMN-E and *IL*-*6* was greater the more severe the symptoms. However, no significant correlation was observed between the number of organs damaged and the maximum serum concentrations of P-AMY and E-1, or the WBC count, which have been considered to be markers of *pancreatitis*. These results suggest that PMN-E and *IL*-*6* concentrations are useful indicators of severity and prognosis and their determination facilitates the selection of appropriate treatment in the early stages of disease to prevent the aggressive progression of acute *pancreatitis*.

11/3,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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11655968 99090641 PMID: 9873955
Beneficial effect of octreotide treatment in acute *pancreatitis* in rats.
Marton J; Szasz Z; Nagy Z; Jarmay K; Takacs T; Lonovics J; Balogh A; Farkas G
Department of Surgery, Albert Szent-Gyorgyi Medical University, Szeged, Hungary.
International journal of pancreatology - official

journal of the International Association of Pancreatology (UNITED STATES) Dec 1998, 24 (3) p203-10, ISSN 0169-4197 Journal Code: 8703511
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
CONCLUSIONS: Octreotide treatment contributes to the regulation of tumor necrosis factor (TNF) production in sodium taurocholate-induced acute necrotizing *pancreatitis* in rats. Owing to its complex effect, octreotide can partially ameliorate the deleterious consequences of acute necrotizing *pancreatitis*. Elevated TNF and interleukin-6 (*IL*-*6*) levels in the peritoneal fluid may be considered a consequence of the activation of peritoneal macrophages. BACKGROUND: The effects of octreotide on exocrine pancreatic function have been investigated in numerous studies, but little attention has been paid to its influence on cytokine production in acute *pancreatitis*. METHODS: Acute *pancreatitis* was induced by the retrograde injection of taurocholic acid into the pancreatic duct in male Wistar rats. Serum amylase activity, wet pancreatic weight/body weight (pw/bw) ratio, and TNF and *IL*-*6* levels were measured. Four micrograms/kg of octreotide was administered subcutaneously at the time of induction of *pancreatitis* and 24 or 48 h later. Rats were sacrificed 6, 24, 48, or 72 h after the operation. RESULTS: The serum amylase level and pancreatic weight to body weight ratio were decreased significantly in the octreotide-treated group. The serum TNF level was decreased significantly in the octreotide-treated group as compared with the control group at 6, 24, and 48 h (0.6 +/- 1.5, 2.0 +/- 3.3, and 0 vs 50 +/- 15.5, 37.5 +/- 18.4, and 13.1 +/- 12.5 U/mL, respectively). The ascites TNF level was decreased to 0 in the octreotide-treated group and was elevated in the control group at 72 h (28.0 +/- 49.0 U/mL). *IL*-*6* production in ascites was extremely high in both groups at 6 h (80,000 +/- 43,817 pg/mL and 58,500 +/- 33,335 pg/mL), but the difference was not significant.

11/3,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11636935 99070730 PMID: 9855088
Interleukins and their antagonists but not TNF and its receptors are released in post-ERP *pancreatitis*.
Messmann H; Vogt W; Falk W; Vogl D; Zirngibl H; Leser H G; Scholmerich J Department of Internal Medicine I, University of Regensburg, Germany. European journal of gastroenterology & hepatology (ENGLAND) Jul 1998, 10 (7) p611-7, ISSN 0954-691X Journal Code: 9000874
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
OBJECTIVE: Usually it is not possible to study the initial systemic response in patients with acute *pancreatitis* in the first hours after onset of the disease. We used postendoscopic retrograde pancreatography (ERP) *pancreatitis* as a model to study cytokine and anticytokine release in the early phase of human acute *pancreatitis*. METHODS: Post-ERP *pancreatitis* was defined as a threefold increase in serum amylase and at least two of the following clinical symptoms: abdominal pain, nausea, vomiting or peritonism 24 h after ERP. Serum levels of pro-inflammatory cytokines interleukin-1beta (IL-1beta), interleukin-6 (*IL*-*6*), interleukin-8 (IL-8), tumour necrosis factor alpha (TNF), as well as endogenous antagonistic mediators of the systemic inflammatory response such as soluble tumour necrosis factor alpha receptors p55 (TNFR p55) and p75 (TNFR p75), and IL-1-receptor antagonist (IL-1-RA) and interleukin-2-receptor (IL-2R) as indicators of lymphocyte activation were measured before and 0, 1, 4, 12, 24 and 48 h after ERP. In nine patients with acute post-ERP *pancreatitis*, these parameters were monitored daily until C-reactive protein (CRP) was within normal ranges and were compared to patients without *pancreatitis* after ERP. RESULTS: IL-1beta was not detectable in five patients with and four patients without post-ERP *pancreatitis* . The values of the remaining patients in both groups were lower than 3.9 pg/ml. IL-8 and IL-1-RA serum concentrations peaked 12 h after ERP (132.9 and 3245.0 pg/ml respectively) compared to patients without post-ERP *pancreatitis* (25.8 and 389.9 pg/ml respectively). The *IL*-*6* concentration increased to 81.6 pg/ml (8.0 pg/ml in control patients) 24 h after ERP, while the peak values for CRP were measured 72 h after ERP (164.0 versus 7.7 mg/l). IL-2R content was maximally elevated 144 h after ERP (688.8 versus 255.9 U/ml), while concentrations of TNF and its receptors showed no significant change over time. CONCLUSION: The initial response of the cytokine network to damage of the human pancreas leading to acute *pancreatitis* includes the release of IL-8 and the IL-1 antagonist IL-1-RA, while IL-1beta is not found in the systemic circulation. The TNF system does not seem to be involved as indicated by the lack of detectable changes in TNF and the soluble TNFR p55 and p75 serum concentrations. Lymphocyte activation as indicated by elevated IL-2R levels occurred days after the initial trauma. Even mild post-ERP *pancreatitis* leads to significant systemic release of cytokines and their biological counterparts.

11/3,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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11634852 99068590 PMID: 9853701
Portal but not peripheral serum levels of interleukin 6 could interfere with glucose metabolism in patients with pancreatic cancer. Fogar P; Basso D; Pasquali C; Piva M G; Brigato L; De Paoli M; Galeotti F ; Corsini A; Plebani M
Dipartimento di Medicina di Laboratorio, Padova, Italy.
Clinica chimica acta; international journal of clinical chemistry (NETHERLANDS) Oct 1998, 277 (2) p181-9, ISSN 0009-8981 Journal Code: 1302422
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Interleukin 6 (*IL*-*6*), an autocrine growth factor for many tumors, seems to favour tumor spread to the liver. Our aims were first to evaluate the pattern of portal and systemic *IL*-*6* levels in patients with pancreatic cancer (PC, n = 18) and chronic *pancreatitis* (CP, n = 22) compared with controls (CS, n = 20); and second, to ascertain whether there was any relation between *IL*-*6* levels and tumor spread or PC-associated Diabetes mellitus. For all subjects, a fasting serum sample was obtained from a cubital vein; a portal serum sample was obtained from nine PC and three CP patients. In cubital and portal sera we measured *IL*-*6*, interleukin 1 beta (IL-1b), CA 19-9, c-reactive protein (CRP) and amylase. Systemic *IL*-*6* levels were significantly higher in PC patients than in CS. In PC, portal *IL*- *6* levels were significantly higher than the corresponding systemic values. The same pattern was found in the three CP patients, whereas IL-1b, CA 19-9, CRP and amylase portal levels were the same as systemic values. No correlation was found between PC stage and systemic or portal *IL*- *6* levels. Portal *IL*-*6* levels were correlated with the corresponding fasting serum glucose values. A significant correlation was found between *IL*-*6* values and CRP, ALT, total bilirubin, GGT and creatinine, but not amylase. In conclusion: (1) Portal *IL*-*6* , which is partly of pancreatic origin, is first metabolised in the liver; (2) Systemic *IL*-*6* reflects hepatic and renal functions rather than local conditions in the pancreas; (3) *IL*-*6* does not appear to influence PC spread; (4) *IL*-*6*, which is released in large amounts by the inflamed pancreas, may contribute to determining diabetes, thus interfering with the signal transducing pathways involved in glucose metabolism in liver cells.

11/3,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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11573887 99005711 PMID: 9789282
[Mechanism of the development of organ failure]
Ura H; Hirata K; Yamaguchi K; Katsuramaki T; Denno R
First Department of Surgery, Sapporo Medical University School of Medicine, Japan.
Nippon Geka Gakkai zasshi (JAPAN) Aug 1998, 99 (8) p485-9, ISSN 0301-4894 Journal Code: 0405405
Document type: Journal Article; Review; Review, Tutorial ; English Abstract
Languages: JAPANESE
Main Citation Owner: NLM
Record type: Completed
Multiple organ failure (MOF) is a critical condition developing in patients with overwhelming bodily injury resulting from major surgical insult, severe trauma, extensive burns, acute *pancreatitis*, and sepsis. It has recently become evident that the host response to such injury is the main pathogenetic factor contributing to the development of MOF. The proinflammatory cytokines tumor necrosis factor (TNF) and interleukin (IL)-1 are known to play a pivotal role in the pathogenetic mechanisms of MOF. In response to bodily injury, macrophages produce and release TNF and IL-1, which subsequently induce the production of other cytokines (*IL*-*6* , IL-8, etc.) and other endogenous chemical mediators. The resultant systemic inflammation may develop into MOF mainly through neutrophil-endothelial cell interaction when the primary injury is overwhelming or a second inflammatory insult such as sepsis triggers an exacerbated inflammation. It has recently been confirmed that the transcription factor NF-kappaB is involved in the up-regulation of a variety of proinflammatory genes and that cell-mediated immunity is down-regulated in the event of major bodily injury through a shift in the balance between T helper 1 (Th1) and Th2 cytokine response patterns. The molecular immunological mechanisms by which these factors participate in the development of MOF should be characterized.

11/3,AB/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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11556847 98448791 PMID: 9775652
Complex treatment of infected necrotizing *pancreatitis*] Inficialodott nekrotizalo *pancreatitis* komplex kezelese. Farkas G; Marton J; Mandi Y; Nagy E; Szederkenyi E
Szent-Gyorgyi Albert Orvostudomanyi Egyetem, Sebeszeti Klinika, Szeged. Orvosi hetilap (HUNGARY) Sep 20 1998, 139 (38) p2235-40, ISSN 0030-6002 Journal Code: 0376412
Document type: Journal Article; Review; Review Literature ; English Abstract

Languages: HUNGARIAN
Main Citation Owner: NLM
Record type: Completed
Pancreatic necrosis associated with septic conditions is the leading cause of mortality in acute *pancreatitis*. Since 1986, 155 patients with infected pancreatic necrosis have been treated. The mean APACHE II score was 18.5 (range 11-32). In all cases, the infected pancreatic necrosis was combined with retroperitoneal abscesses. The surgical treatment was performed on average 18.5 days (range 8-25 days) after the onset of acute *pancreatitis*. The operative management consisted of wide-ranging necrosectomy in the total affected area, combined with widespread lavage and suction drainage. In 69 of the 155 cases (45%), some other surgical intervention (distal pancreatic resection, splenectomy, cholecystectomy, sphincteroplasty or colon resection) was also performed. Following surgery supportive therapy was applied in all patients, which also consisted orf immunonutrition (glutamine and arginine supplementation) and modification of cytokine production by petoxyfillin and dexamethasone from 1992. TNF and *IL*-*6* serum levels were measured by ELISA and in vitro stimulation of leukocytes were induced by E. coli LPS. Following surgery, continuous lavage and suction drainage were applied for an average of 41.5 days (range 21-90 days), with an average of 9.5 (range 5-20) litres of saline per day. The bacteriologic findings revealed mainly enteral bacteria, but Candida infection was also frequently detected. The incidence of fungal infection was 20%. Thirty-two patients (21%) had to undergo reoperation. The cytokine production capacity (TNF and *IL*-*6*) was shown to correlate with the prognosis. As a consequence of pentoxifyllin and dexamethasone therapy, the TNF production generally dropped to the normal level. The overall hospital mortality was 6.4% (10 patients died). In our experience, infected pancreatic necrosis responds well to aggressive surgical treatment, continuous, long-standing lavage and suction drainage, together with supportive therapy consisting of immunonutrition and modification of cytokine production, combined with adequate antibiotic and antifungal medication.

11/3,AB/7 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11553775 98445668 PMID: 9772491
Changes of inflammatory mediator in aucte necrotizing *pancreatitis* and effect of somatostatin analogne stilamin in rats]
Zhang Q; Cai D; Wu S
Department of Surgery, Huashan Hospital, Shanghai Medical University. Zhonghua yi xue za zhi (CHINA) May 1997, 77 (5) p355-8, ISSN 0376-2491 Journal Code: 7511141
Document type: Journal Article ; English Abstract
Languages: CHINESE
Main Citation Owner: NLM
Record type: Completed
OBJECTIVES: To investigate the changes of inflammatory mediator in ANP and to explore the effectiveness of stilamin treatment on ANP. METHODS: SD rats were divided into experimental groups (ANP = 12, ANP + NS = 10, ANP + Stilamin = 12) and control group (NC = 10). Rat ANP models were made by retrograde injection of 3.5% sodium taurocholate 2.5 ml/kg into the pancreatic duct, and treated by intravenous injection of stilamin 84 micrograms.kg-1.d-1 or same amount of normal saline respectively. Serum IL-1 beta, *IL*-*6*, IL-12, TNF-alpha, PLA2, amylase and endotoxin were examined. Finally, the morphology and pathology of the pancreas, liver, lung, heart and kidney, as well as the electronmicroscopical investigation of liver and lung cells were observed. RESULTS: The mean IL-1 beta, *IL*-*6* , IL-12, TNF-alpha, in the ANP and ANP + NS group were higher than those in the ANP + stilamin group and Control group ($P < 0.01$). The endotoxin, amlyase, PLA2 level in the ANP + stilamin group was lower than those in the ANP and ANP + NS groups ($P < 0.001$). ANP significantly increased white blood cell chemotax and infiltration, as well as hemorrhagic focus in liver, kidney and lung, but the pathological examinations of the liver, pancreas, lung and kidney demonstrated much less destruction in the stilamin group. CONCLUSIONS: Inflammatory cytokins play an essential role to induce pathophysiological process of ANP, and its related MODS may be effectively prevented and treated by stilamin.

11/3,AB/8 (Item 8 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11538370 98429688 PMID: 9755294
Heparin and nitric oxide treatment in experimental acute *pancreatitis* in rats.
Dobosz M; Wajda Z; Hac S; Mysliwska J; Mionskowska L; Bryl E; Roszkiewicz A; Mysliwski A
IInd Department of Surgery, Medical University of Gdansk, Gdansk, Poland. Forum (Genoa, Italy) (ITALY) Jul-Sep 1998, 8 (3) p303-10, ISSN 1121-8142 Journal Code: 9315183
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The aim of this study was to investigate the impact of L-arginine (nitric oxide synthase substrate), L-NG-nitro-L-arginine (nitric oxide synthase inhibitor),

and heparin on the pancreas microcirculation, serum *IL*- *6* level and microscopic alterations of the pancreas in acute *pancreatitis* in rats. Acute *pancreatitis* was induced by 4 i.p. injections of cerulein (15mg/kg). Microcirculatory values were measured by means of laser Doppler flowmetry 5 h after the first cerulein injection. Remarkable histopathological changes in the pancreas, including parenchymal necrosis, an elevation of serum *IL*-*6* level, and a significant drop of pancreatic capillary perfusion was observed in rats with nitric oxide synthase inhibition. L-arginine improved the pancreatic microcirculation but worsened the microscopic alterations within the pancreas. Heparin had a beneficial effect on the microcirculatory values, serum *IL*-*6* concentration, and morphologic changes. Authors conclude that inhibition of nitric oxide synthase aggravates acute *pancreatitis* . L-arginine treatment improves pancreatic perfusion but potentiates morphological alterations. Heparin, improving the microcirculation and inflammatory changes within the pancreatic gland, may be considered as a promising therapeutic agent in acute *pancreatitis*.

11/3,AB/9 (Item 9 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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11504244 98389559 PMID: 9724166
Diagnostic relevance of interleukin pattern, acute-phase proteins, and procalcitonin in early phase of post-ERCP *pancreatitis*. Oezcueruemez-Porsch M; Kunz D; Hardt P D; Fadgyas T; Kress O; Schulz H U; Schnell-Kretschmer H; Temme H; Westphal S; Luley C; Kloer H U Third Department of Internal Medicine, University of Giessen, Germany. Digestive diseases and sciences (UNITED STATES) Aug 1998, 43 (8) p1763-9, ISSN 0163-2116 Journal Code: 7902782
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Post-endoscopic retrograde cholangiopancreatography (ERCP) *pancreatiti* *s* has been suggested as a model for acute *pancreatitis* (AP), which allows evaluation of early alterations in the time course of the disease. The influence of the clinical course on procalcitonin (PCT), serum amyloid A (SAA), and several proinflammatory and inhibitory cytokines was evaluated in patients with AP following ERCP. Blood samples were prospectively collected from patients undergoing ERCP. The incidence of ERCP-induced pancreatic damage, defined as abdominal complaints, a threefold increase of serum lipase, and elevation of CRP from <10 to >20 mg/liter was 12.8% (12/94). Only mild clinical courses of acute *pancreatitis* were observed. PCT significantly increased in subjects with post-ERCP *pancreatitis* after 24 hr. However, PCT levels did not exceed 0.5 ng/ml in any patient. Interleukin-1 receptor antagonist (IL-1RA) began to differ from baseline 2 hr after ERCP, followed by interleukin-6 (*IL*- *6* , 6 hr), solubilized tumor necrosis factor-alpha receptor II (sTNF-alphaRII, 24 hr) and SAA (24 hr). Interleukin 10 (IL-10) showed marked interindividual variations with no obvious peak. Among all parameters evaluated, only peak values of *IL*-*6* and IL-10 showed significant correlations with the reported pain score (r2 = 0.62/0.78), degree of ampullar irritation (r2 = NS/0.87), and the duration of ERCP (r2 = 0.58/0.76). No correlation was found with the volume of the injected contrast agent. We conclude that IL-10 and *IL*-*6* appear to be useful to monitor patients after ERCP. The absence of any PCT elevation in the present study is in accordance with the clinical course of the patients who suffered from mild pancreatic damage without systemic or infectious complications.

11/3,AB/10 (Item 10 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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11438718 98321618 PMID: 9660181
Clusterin overexpression in rat pancreas during the acute phase of *pancreatitis* and pancreatic development.
Calvo E L; Mallo G V; Fiedler F; Malka D; Vaccaro M I; Keim V; Morisset J ; Dagorn J C; Iovanna J L
Departement de Medecine, Faculte de Medecine, Universite de Sherbrooke, Qc, Canada.
European journal of biochemistry / FEBS (GERMANY) Jun 1 1998, 254 (2) p282-9, ISSN 0014-2956 Journal Code: 0107600
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Molecular mechanisms associated with apoptosis in pancreas remain largely unknown. Clusterin mRNA is induced in several tissues in response to most apoptotic stimuli. In these tissues, clusterin has an antiapoptotic activity. The aim of this work was to test whether clusterin, which is not expressed in normal pancreas, was induced in pancreas during *pancreatitis* and pancreatic development. Clusterin mRNA levels were strongly increased 6 h after *pancreatitis* induction. Maximal expression happened between 24-48 h and decreased progressively to undetectable levels at day 5. Clusterin mRNA was expressed with similar intensity in oedematous caerulein-induced *pancreatitis* and in response to various degrees of necrohaemorrhagic

taurocholate-induced *pancreatitis*, indicating a maximal gene activity in all types of *pancreatitis* ; in situ hybridization showed that the acinar cells and some ducts expressed clusterin mRNA. A single band of about 35-38 kDa was detected by western blot in pancreatic homogenates and in pancreatic juice from rats with acute *pancreatitis* , but not from control rats. Clusterin mRNA expression was strong in late fetal life and remains high until day 11 post-partum, then decreased progressively with a minimum from 35 to 90 days post-partum. Clusterin mRNA levels were strongly induced in pancreatic acinar AR4-2J cells in response to various apoptotic stimuli (i.e., cycloheximide, staurosporine, ceramide and H2O2) but not with interleukin (IL)-1, IL-4 or *IL*-*6* or heat shock, which do not induce apoptosis in AR4-2J cells. In conclusion, we demonstrated that clusterin is synthesized and released by the pancreas. Its strong expression during acute *pancreatitis* suggests its involvement in the pancreatic response to injury. Clusterin is also induced during pancreatic development. Because these situations are associated with apoptosis and clusterin was shown to protect against apoptosis, we speculate that clusterin could be involved in the control of acinar cell apoptosis.

11/3,AB/11 (Item 11 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11428816 98311333 PMID: 9648999
Value of early blood Th-1 cytokine determination in predicting severity of acute *pancreatitis*.
Heresbach D; Letourneur J P; Bahon I; Pagenault M; Guillou Y M; Dyard F; Fauchet R; Malledant Y; Bretagne J F; Gosselin M
Dept. of Hepato-Gastro-Enterology, Pontchaillou University Hospital, Rennes, France.
Scandinavian journal of gastroenterology (NORWAY) May 1998, 33 (5) p554-60, ISSN 0036-5521 Journal Code: 0060105
Document type: Clinical Trial; Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
BACKGROUND: Early evaluation of the severity of acute *pancreatitis* (AP) requires measurement of many variables within 48 h after admission. Septic complications (SC) are frequent, and preliminary studies have highlighted the value of prophylactic antibiotherapy; however, single and reliable predictive markers of sepsis are not yet available. The aim of this study was to assess the value of determining early blood Th-1 cytokines and their natural antagonists (interleukin-6 (*IL*-*6*), IL-1, IL-1ra, and the soluble form of tumor necrosis factor (sTNF) receptors RI and RII) to predict the severity and SC during AP. METHODS: Thirty-seven patients with AP were prospectively included; 25 of them had severe AP, including 8 with SC. Serum cytokines were measured 48 h and 72 h after the onset of AP with an enzyme-linked immunosorbent assay. The optimal severity or SC diagnostic thresholds was determined using receiver operative curves. RESULTS: Severe AP in accordance with the Atlanta criteria were better predicted by C-reactive protein and *IL*-*6* serum determination, albeit these levels could not predict absolutely the death of two patients. In severe AP cases (n = 25) the IL-1 to IL-1-ra ratio was lower in cases further complicated by sepsis ((6+/-4) 10(-3) versus (34+/-13) 10(-3), P < 0.05); moreover, sTNF RI (2497+/-270 pg/ml versus 2133+/-611 pg/ml, P < 0.05) and RII (3751+/-400 pg/ml versus 3045+/-509 pg/ml, P < 0.05) were higher in AP characterized by further SC. The IL-1 to IL-1-ra ratio and IL-1 concentration were dramatically decreased within the first 48 h ((0.4+/-0.4) 10(-3) versus (30+/-11) 10(-3), P < 0.05, and 0.3+/-0.3 versus 15+/-3 ng/l, P < 0.05) in patients with further infection of the pancreatic necrosis (n = 3). The SC diagnosis was better anticipated by an IL-1 to IL-1-ra ratio lower than 5 x 10(-3) or by an sTNF RI higher than 1750 pg/ml and sTNF RII higher than 2750 pg/ml, and the infection of the pancreatic necrosis by an IL-1 concentration <2 ng/l or an IL-1 to IL-1-ra ratio <2 x 10(-3). CONCLUSION: Besides severity markers, IL-1, IL-1-ra, and sTNF RI and RII should be considered in base-line AP assays and, if confirmed by larger studies, could help to screen patients at risk for SC and candidates for prophylactic antibiotherapy with a good negative predictive value.

11/3,AB/12 (Item 12 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11362131 98242670 PMID: 9583368
Glutamine-supplemented total parenteral nutrition reduces blood mononuclear cell interleukin-8 release in severe acute *pancreatitis*.
de Beaux A C; O'Riordain M G; Ross J A; Jodozi L; Carter D C; Fearon K C University Department of Surgery, Royal Infirmary, Edinburgh, United Kingdom.
Nutrition (Burbank, Los Angeles County, Calif.) (UNITED STATES) Mar 1998, 14 (3) p261-5, ISSN 0899-9007 Journal Code: 8802712 Document type: Clinical Trial; Journal Article; Randomized Controlled Trial
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Glutamine, a conditionally essential amino acid, is

important for immune function. It is now being formulated for incorporation into total parenteral nutrition (TPN). The aims of this study were to examine the effect of glutamine administration on lymphocyte proliferation and proinflammatory cytokine release in patients with severe acute *pancreatitis* . Fourteen patients were randomized (in a double-blind fashion) to receive either conventional or isocaloric, isonitrogenous glutamine-supplemented (0.22 g glutamine x kg(-1) x d(-1) as glycyl-glutamine) TPN for 7 d. DNA synthesis (index of lymphocyte proliferation) and the 24-h release of tumor necrosis factor (TNF), interleukin (*IL*)-*6*, and IL-8 from peripheral blood mononuclear cells were measured in vitro on days 0, 4, and 7. Thirteen patients completed the study protocol (6 glutamine TPN, 7 conventional TPN). Glutamine supplementation increased median DNA synthesis by 3099 cpm over the study period against 219 cpm in the conventional group (increase not significantly different between the two groups) . Glutamine supplementation did not significantly influence TNF or *IL*-*6* release, but, in contrast, median IL-8 release was reduced by day 7 in the glutamine group while it was increased in the conventional group (-17.7 ng/mL (median change over study period) versus +43.3 ng/mL, respectively; P=0.045). Small patient numbers and substantial interindividual variation limit the conclusions, but there is a trend for the glutamine group to have improved lymphocyte proliferation, and in the case of IL-8, reduced proinflammatory cytokine release.

11/3,AB/13 (Item 13 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11342288 98222344 PMID: 9561559
Beneficial effects of pentoxifylline treatment of experimental acute *pancreatitis* in rats.
Marton J; Farkas G; Takacs T; Nagy Z; Szasz Z; Varga J; Jarmay K; Balogh A; Lonovics J
Department of Surgery, Albert Szent-Gyorgyi Medical University, Szeged, Hungary.
Research in experimental medicine. Zeitschrift fur die gesamte experimentelle Medizin einschliesslich experimenteller Chirurgie (GERMANY) 1998, 197 (5) p293-9, ISSN 0300-9130 Journal Code: 0324736
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The purposes of this study were to determine the tumor necrosis factor (TNF) and interleukin-6 (*IL*-*6*) levels after the induction of acute necrotizing *pancreatitis* , and to establish the effects of pentoxifylline on cytokine production. METHODS: acute *pancreatitis* was induced by the retrograde injection of 200 microliters taurocholic acid into the pancreatic duct in male Wistar rats. The serum amylase activity, the wet pancreatic weight/body weight ratio, and the TNF and *IL*- *6* levels were measured. Seven mg/kg pentoxifylline were administered intraperitoneally at the time of operation 6, 12 or 24 h later. Rats were killed 6, 24, 48 or 72 h after the operation. RESULTS: the TNF bioassay revealed high levels of TNF (30.2 +/- 5.4 U/ml, 35.0 +/- 5.0 U/ml and 36.6 +/- 6.0 U/ml) in the control group at 6, 24 and 48 h and (54.1 +/- 20 U/ml and 10.9 +/- 4.2 U/ml) in the pentoxifylline-treated group at 6 and 24 h, respectively, whereas the level had decreased to zero in the pentoxifylline-treated group at 48 h. The *IL*-*6* bioassay likewise demonstrated high levels of *IL*-*6* in the control group at 48 h and in the pentoxifylline-treated group at 6 and 24 h, and markedly decreased levels in the pentoxifylline-treated group at 48 h (7083 +/- 2844 pg/ml, 6463 +/- 1307 pg/ml, 10,329 +/- 5571 pg/ml vs 137.5 +/- 85.5 pg/ml, respectively, P < 0.05). The high mortality observed in the *pancreatitis* group (43%) was decreased by pentoxifylline administration to 11%. CONCLUSION: these results demonstrate that pentoxifylline very effectively inhibits cytokine production in acute *pancreatitis*.

11/3,AB/14 (Item 14 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11310118 98189410 PMID: 9530927
Experimental acute *pancreatitis* results in increased blood-brain barrier permeability in the rat: a potential role for tumor necrosis factor and interleukin 6.
Farkas G; Marton J; Nagy Z; Mandi Y; Takacs T; Deli M A; Abraham C S Department of Surgery, Albert Szent-Gyorgyi University Medical School, Szeged, Hungary. farkas@surg.szote.u-szeged.hu
Neuroscience letters (IRELAND) Feb 20 1998, 242 (3) p147-50, ISSN 0304-3940 Journal Code: 7600130
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Pancreatic encephalopathy is a severe complication of acute *pancreatitis* . Proinflammatory cytokines may play a role in the development of multi-organ failure during *pancreatitis* . In the present study, we measured the changes in the blood-brain barrier (BBB) permeability concomitantly with the determination of serum tumor necrosis factor (TNF) and interleukin-6 (*IL*-*6*) levels in rats before, as well as 6, 24 and 48 h after the beginning of

intraductal taurocholic acid-induced acute *pancreatitis* . Cytokine concentrations were measured in bioassays with specific cell lines (WEHI-164 for TNF and B-9 for *IL*-*6*), while the BBB permeability was determined for a small (sodium fluorescein, molecular weight (MW) 376 Da), and a large (Evans' blue-albumin, MW 67000 Da) tracer by spectrophotometry in the parietal cortex, hippocampus, striatum, cerebellum and medulla of rats. The serum TNF level was significantly ($P < 0.05$) increased 6 and 24 h after the induction of *pancreatitis*, while the *IL*-*6* level increased after 24 and 48 h. A significant ($P < 0.05$) increase in BBB permeability for both tracers developed at 6 and 24 h in different brain regions of animals with acute *pancreatitis*. We conclude that cytokines, such as TNF and *IL*-*6* , may contribute to the vasogenic brain edema formation during acute *pancreatitis*.

11/3,AB/15 (Item 15 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11211596 98088461 PMID: 9427022
Surgical treatment for severe acute *pancreatitis*: factors which affect the surgical results.
Yamamoto M; Takeyama Y; Ueda T; Hori Y; Nishikawa J; Saitoh Y First Department of Surgery, Kobe University School of Medicine, Japan. Hepato-gastroenterology (GREECE) Nov-Dec 1997, 44 (18) p1560-4, ISSN 0172-6390 Journal Code: 8007849
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Surgical treatment for severe acute *pancreatitis* has not yet yielded satisfactory results. Several factors which might affect the surgical results of severe acute *pancreatitis* were analyzed in this study. The presence or absence of infection was not important as a factor determining the surgical results. The severity scores and some biochemical parameters such as CRP, *IL*-*6*, PMN-E, HGF seemed to be closely related to surgical results. It was likely that a significant decrease in lymphocyte counts in the blood on admission was closely related to the prognosis of the surgical patients. Timing and procedures for surgery should be more seriously considered in the treatment for patients with such poor general conditions.

11/3,AB/16 (Item 16 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11196035 98072634 PMID: 9408354
Plasma levels of TNF and *IL*-*6* following induction of acute *pancreatitis* and pentoxifylline treatment in rats.
Marton J; Farkas G; Nagy Z; Takacs T; Varga J; Szasz Z; Balogh A; Lonovics J
Department of Surgery, Albert Szent-Gyorgyi Medical School, Szeged, Hungary.
Acta chirurgica Hungarica (HUNGARY) 1997, 36 (1-4) p223-5, ISSN 0231-4614 Journal Code: 8309977
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Activation of cytokine cascade is a decisive factor in determining the pathobiology of different inflammatory processes including acute *pancreatitis* . The purposes of this study were to determine the TNF and *IL*-*6* levels after the induction of acute necrotizing *pancreatitis* , and to establish the effects of pentoxifylline on the cytokine production and the severity of *pancreatitis* . Acute necrotizing *pancreatitis* was induced by the retrograde injection of 200 microliters taurocholic acid into the pancreatic duct in male Wistar rats. TNF was titrated in a bioassay on cell line WEHI clone 164. *IL*- *6* was measured via its proliferative action on the *IL*-*6* dependent mouse hybridoma cell line B-9. Seven mg/kg pentoxifylline was administered intraperitoneally at the time of operation and/or 24 hours later. Rats were sacrificed, 48 or 72 hours after the operation. The TNF bioassay revealed high levels of TNF (36.6 +/- 6.0 U/ml) in the control group whereas levels decreased to zero in the pentoxifylline-treated group. The *IL*-*6* bioassay likewise demonstrated high levels of *IL* -*6* in the control group and markedly decreased levels in the pentoxifylline treated group (7083 +/- 2844 pg/ml, 6463 +/- 1307 pg/ml vs. 137.5 +/- 85.5 pg/ml, respectively, $p < 0.05$). The high mortality observed in the control group (43%) was sharply decreased by pentoxifylline administration to 11%. The data suggest that pentoxifylline is capable of modifying the cytokine production after 48 hours of induction of acute *pancreatitis*.

11/3,AB/17 (Item 17 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11196023 98072622 PMID: 9408342
The effects of glucocorticoids and a glucocorticoid antagonist (RU 38486) on experimental acute *pancreatitis* in rat.
Lazar G; Varga J; Lazar G; Duda E; Takacs T; Balogh A; Lonovics J Department of Surgery, Albert Szent-Gyorgyi Medical University, Hungary. Acta

chirurgica Hungarica (HUNGARY) 1997, 36 (1-4) p190-1, ISSN 0231-4614 Journal Code: 8309977
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The effects of glucocorticoids on acute *pancreatitis* are a matter of dispute. In animal experiments, dexamethasone and hydrocortisone significantly decreased the serum amylase activities 8 hours after the induction of *pancreatitis* . In the dexamethasone treated group, the serum *IL*-*6* level was significantly decreased at 4 and 8 hours, while in the hydrocortisone treated group, all the *IL*-*6* values were significantly diminished vs. the control group. As compared to the control, a glucocorticoid antagonist (RU 38486) did not influence the serum amylase activity, but significantly increased the serum *IL*-*6* level. These results suggest that glucocorticoids may play a role in the control of *pancreatitis* caused by inhibition of cytokine production.

11/3,AB/18 (Item 18 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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11195979 98072578 PMID: 9408298
Relevance of cytokine production to infected pancreatic necrosis. Farkas G; Nagy Z; Marton J; Mandi Y
Department of Surgery, Albert Szent-Gyorgyi Medical University, Szeged, Hungary.
Acta chirurgica Hungarica (HUNGARY) 1997, 36 (1-4) p86-8, ISSN 0231-4614 Journal Code: 8309977
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The purpose of this study was to evaluate the role of cytokines in septic conditions following acute *pancreatitis* and to elaborate a new strategy in the treatment. Increased TNF and *IL*-*6* serum levels were found in 30% of the patients (n = 40), while the *IL*-*6* level was elevated in all of them. There was a positive correlation between the serum *IL*-*6* and sICAM-1 levels. The in vitro TNF and *IL*-*6* producing capacities were initially higher in the study group, but decreased on subsequent days, especially in fatal cases (n = 3). Administration of pentoxifylline [PTX] (400 mg/day) to septic patients following necrotizing *pancreatitis* resulted in TNF and *IL*-*6* production similar to that observed in control donors. The level of sICAM-1 also decreased following PTX therapy. These results suggest that cytokines produced by activated leucocytes are important in the pathogenesis of infected pancreatic necrosis, and their inhibition might be of therapeutic advantage.

11/3,AB/19 (Item 19 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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11168361 98044543 PMID: 9424855
Microcirculation disorders of the pancreas in cerulein induced acute *pancreatitis* in rats with regard to nitrogen oxide and heparin] Zaburzenia mikrokrazenia w trzustce w ceruleinowym ostrym zapaleniu trzustki u szczurow z uwzglednieniem wplywu tlenku azotu i heparyny. Hac D S; Mionskowska L; Dobrowolski S; Dymecki D; Makarewicz W; Wajda Z II Katedry i Kliniki Chirurgii Ogolnej, Gastroenterologicznej i Endokrynologicznej Akademii Medycznej w Gdansku.
Wiadomosci lekarskie (Warsaw, Poland - 1960) (POLAND) 1997, 50 Suppl 1 Pt 2 p108-14, ISSN 0043-5147 Journal Code: 9705467
Document type: Journal Article ; English Abstract
Languages: POLISH
Main Citation Owner: NLM
Record type: Completed
Microcirculatory disturbance may play an important role in the development of severe *pancreatitis*, leading the edematous form of the disease to the necrosis. The aim of this study was to investigate the impact of L-arginine (nitric oxide donor), L-NN (NO synthase inhibitor), and heparin on the pancreas microcirculation, serum interleukin-6 level and microscopic alterations of the pancreas in acute *pancreatitis* in rats. METHODS: Acute *pancreatitis* was induced in 72 rats by four intraperitoneal injections of cerulein (CN) (15 micrograms/kg body weight). Microcirculatory values was measured by means of laser Doppler flowmetry five hours after the first cerulein injection. The animals were divided into the following groups (12 rats each), according to the kind of treatment: Group 1 (CN), Group 2 (CN + L-NNA), Group 3 (CN + L-arginine), Group 4 (CN + Heparin), Group 5 (Control), Group 6 (L-NNA), Group 7 (L-arginine), Group 8 (Heparin). RESULTS: Remarkable morphologic changes in the pancreas including parenchymal necrosis, an elevation of serum *IL* -*6* level, and significant drop of pancreatic capillary perfusion was observed in rats with NO synthase inhibition. L-arginine improved the pancreatic microcirculatory but worsened the microscopic alteration within the pancreas. Heparin had a beneficial effect on the microcirculatory values, serum *IL*-*6* concentration, and morphologic changes. CONCLUSIONS: Acute *pancreatitis* causes microcirculatory disturbance within the pancreatic gland. The inhibition of NO synthase aggravates AP. L-arginine treatment improves pancreatic perfusion but potentiates morphologic alterations. Heparin has beneficial impact on AP, it improves the microcirculation and inflammatory changes within the pancreatic gland.

11/3,AB/20 (Item 20 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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11137274 98012929 PMID: 9352880
Gene targeting demonstrates additive detrimental effects of interleukin 1 and tumor necrosis factor during *pancreatitis*.
Denham W; Yang J; Fink G; Denham D; Carter G; Ward K; Norman J Department of Surgery and the Pancreas Study Group, University of South Florida, Tampa 33612, USA.
Gastroenterology (UNITED STATES) Nov 1997, 113 (5) p1741-6, ISSN 0016-5085 Journal Code: 0374630
Comment in Gastroenterology. 2000 Sep;119(3) 881-2; Comment in PMID 11023363
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
BACKGROUND & AIMS: During severe *pancreatitis* , interleukin (IL)-1beta and tumor necrosis factor (TNF)-alpha are produced in large quantities. The aim of this study was to determine whether either one plays a more dominant role and if their detrimental effects are additive. METHODS: Necrotizing *pancreatitis* was induced in transgenic (-/-) knockout mice deficient in either IL-1 type 1 receptors, TNF type 1 receptors, or both IL-1 and TNF type 1 receptors. Wild-type mice served as controls. Mortality was assessed for 10 days. Additional animals were killed on days 0, 1, 2, 3, and 4 for determination of *pancreatitis* severity. RESULTS: All three knockout groups showed decreased amylase and lipase, histological score, serum *IL*-*6*, and mortality compared with wild-type groups. Animals devoid of receptors for both cytokines showed improved survival and decreased *IL*-*6* levels compared with those devoid of either IL-1 or TNF receptors individually, yet they failed to show a further decrease in *pancreatitis* severity. CONCLUSIONS: Preventing the activity of IL-1beta or TNF-alpha has a nearly identical beneficial effect on the severity and mortality of acute *pancreatitis* . Preventing the activity of both cytokines concurrently has no additional effect on *pancreatitis* severity but further attenuates the systemic stress response and is associated with an additional but modest decrease in mortality.

11/3,AB/21 (Item 21 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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11079111 97434266 PMID: 9288152
An ETa/ETb endothelin antagonist ameliorates systemic inflammation in a murine model of acute hemorrhagic *pancreatitis*.
Todd K E; Lewis M P; Gloor B; Lane J S; Ashley S W; Reber H A Department of Surgery, Sepulveda VA Medical Center, Calif., USA. Surgery (UNITED STATES) Aug 1997, 122 (2) p443-9; discussion 449-50, ISSN 0039-6060 Journal Code: 0417347
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
BACKGROUND: Endothelin peptides are polykines with strong vasoconstrictor properties. We have previously shown that endothelin antagonism (PD145065) reduces the local severity of acute *pancreatitis*. We now investigated the effect of endothelin antagonism on systemic inflammation in a model of acute hemorrhagic *pancreatitis*. METHODS: Forty-two mice were divided into four groups. Group 1 was fed standard food plus PD145065 every 8 hours. Group 2 was fed a choline-deficient ethionine (CDE) supplemented diet and given saline every 8 hours. Group 3 was fed a CDE diet and treated with PD145065 every 8 hours from initiation of diet. Group 4 was fed a CDE diet and given PD145065 from 48 hours after initiation of diet. Animals were killed at 70 hours. Serum was collected. Pancreata and lung tissue were harvested. RESULTS: Histology score, serum amylase level, lung myeloperoxidase, and interleukin (IL)-10 were all significantly reduced in both treatment groups (groups 3 and 4) ($p < 0.05$). *IL*-*6* levels were reduced in group 3 only ($p < 0.05$). The mortality rate did not differ among any of the groups. CONCLUSIONS: Endothelin antagonism decreased the severity of acute *pancreatitis* and reduced markers of systemic inflammation. Late treatment at 48 hours failed to prevent the rise in *IL*-*6*. Mortality rates were unaffected by treatment.

11/3,AB/22 (Item 22 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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11079094 97434249 PMID: 9288135
Evidence for an unknown component of pancreatic ascites that induces adult respiratory distress syndrome through an interleukin-1 and tumor necrosis factor-dependent mechanism.
Denham W; Yang J; Norman J
Department of Surgery, University of South Florida, Tampa 33612, USA. Surgery (UNITED STATES) Aug 1997, 122 (2) p295-301; discussion 301-2 , ISSN 0039-6060 Journal Code: 0417347
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

BACKGROUND: The development of acute respiratory distress syndrome (ARDS) during acute *pancreatitis* is associated with interleukin (IL)-1 and tumor necrosis factor (TNF) gene expression within the pulmonary parenchyma. Although activated pancreatic enzymes have been thought to mediate *pancreatitis* -induced ARDS, they are not capable of inducing cytokine production in vitro. We hypothesized that IL-1 and TNF production in the lungs is essential to the development of ARDS and is induced by a mediator released from the inflamed pancreas. METHODS: Pancreatic ascites was obtained from rats after induction of bile-salt *pancreatitis*, cultured, and assayed for IL-1, TNF, *IL*-*6* , IL-8, IL-10, interferon-gamma, and endotoxin. Sterile, cytokine-free ascites or saline (control) was subsequently administered intravenously (20 ml/kg) to healthy rats and to IL-1 R1 or TNF R1 knockout mice. RESULTS: Animals administered intravenous ascites had a 30-fold rise in pulmonary IL-1 and TNF mRNA, as well as increased alveolar leukocytes and protein. Knockout animals devoid of active IL-1 or TNF receptors failed to develop increased alveolar protein or leukocytes. CONCLUSIONS: A component of pancreatic ascites other than activated enzymes, bacteria, or inflammatory cytokines is capable of inducing ARDS in healthy animals. The mechanism appears to be directly attributable to the activity of pulmonary IL-1 and TNF.

11/3,AB/23 (Item 23 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10900149 97252114 PMID: 9157344
Cytokines in experimental acute *pancreatitis*]
Citokinek kiserletes akut pancreatitisben.
Marton J; Farkas G; Nagy Z; Takacs T; Jarmay K; Varga J; Balogh A Szent-Gyorgyi Albert Orvostudomanyi Egyetem, Szeged, Sebeszeti Klinika. Orvosi hetilap (HUNGARY) Mar 23 1997, 138 (12) p739-42, ISSN 0030-6002 Journal Code: 0376412
Document type: Journal Article ; English Abstract
Languages: HUNGARIAN
Main Citation Owner: NLM
Record type: Completed
The authors induced acute necrotizing *pancreatitis* in Wistar rat by intraductal injection of taurocholic acid (150 microliters or 200 microliters 6%). Plasma values of amylase, TNF, *IL*-*6* levels and wet pancreas weight/body weight ratio have been determined. Histologic analysis of pancreas proved severe acute necrotizing *pancreatitis* with microabscess formation and beginning respiratory distress syndrome was observed in the lungs, TNF and *IL*-*6* levels increased significantly after administration of 200 microliters 6% taurocholic acid. The authors emphasise the importance of cytokines in the development of acute necrotizing *pancreatitis*.

11/3,AB/24 (Item 24 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10859872 97211299 PMID: 9058297
Hepatocyte growth factor in assessment of acute *pancreatitis*: comparison with C-reactive protein and interleukin-6.
Ueda T; Takeyama Y; Hori Y; Nishikawa J; Yamamoto M; Saitoh Y First Department of Surgery, Kobe University School of Medicine, Japan. Journal of gastroenterology (JAPAN) Feb 1997, 32 (1) p63-70, ISSN 0944-1174 Journal Code: 9430794
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Serum levels of hepatocyte growth factor (HGF), C-reactive protein (CRP), and interleukin-6 (*IL*-*6*) were determined at the time of admission in 38 patients with acute *pancreatitis* . The clinical utility of HGF for the detection of severe *pancreatitis* and for predicting prognosis, bacterial infection (infected pancreatic necrosis or sepsis), and organ dysfunction (liver, kidney, and lung) during the clinical course of acute *pancreatitis* was compared with the clinical utility of CRP and *IL*-*6* by analysis of receiver operator characteristic (ROC) curves. The optimum cutoff levels of HGF for severity, prognosis, infection, hepatic dysfunction, renal dysfunction, and respiratory dysfunction were 0.9, 1.1, 1.0, 1.1, 1.1, and 1.0 ng/ml, respectively. HGF was as useful as CRP and more useful than *IL*- *6* for detection of severe *pancreatitis* and for predicting hepatic dysfunction. Moreover, HGF was more useful than CRP or *IL*- *6* for predicting prognosis, renal dysfunction, and respiratory dysfunction. However, for predicting infection, CRP was more useful than HGF. These results suggest that serum HGF levels on admission may be a useful new clinical parameter for determining the prognosis of acute *pancreatitis* and that HGF may be closely related to the organ dysfunction of acute *pancreatitis*.

11/3,AB/25 (Item 25 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10851319 97202667 PMID: 9133100
Cellular and humoral functions in acute *pancreatitis*]
Zellulare und humorale Funktionen bei der akuten

Pankreatitis. Schulz H U; Schmidt D; Kunz D; Pross M; Gerber A; Weiss G; Sokolowski A; Struy H; Lippert H
Klinik fur Chirurgie, Otto von Guericke-Universitat Magdeburg, Deutschland.
Wiener medizinische Wochenschrift (1946) (AUSTRIA) 1997, 147 (1) p10-3, ISSN 0043-5341 Journal Code: 8708475
Document type: Journal Article ; English Abstract
Languages: GERMAN
Main Citation Owner: NLM
Record type: Completed
Infectious complications are the leading cause of death in acute *pancreatitis* . Individual factors of immune defence could be of significance, whether or not a patient develops a severe course with infectious complications. In a prospective 5-year trial including 72 patients, we investigated 29 cellular and humoral markers of the body's defence system for their potential to indicate the severity and course of acute *pancreatitis* . Complement factors C3 and C4 as well as immunoglobulins IgG, IgM and IgA were normal, in general. Measurable levels of IL-1 alpha, IL-1 beta, IL-2 and sIL-2R could be detected only occasionally. Values of alpha 1-AT, TNF-alpha, TNF alpha-Rp75, neopterin, sICAM-1, IL-8, IL-1RA and sIL-6R did not correlate with a severe course. Due to the high magnitude of increase, CRP, *IL*-*6* and granulocyte elastase were the best indicators of the inflammatory process. Delayed-type hypersensitivity response was the only early predictor of a severe course. It was superior over other cellular markers such as monocyte count or CD4+/CD8+ ratio. In vitro function of polymorphonuclear granulocytes (PMN) was not adequate to the severity of the disease already during the first week of illness. During further course, PMN motility and capacities to produce reactive oxygen species even worsened. The compromized PMN function could explain the frequent development of infectious complications in patients suffering from severe *pancreatitis* . These results should encourage new concepts of infection prophylaxis using stimulants of cellular defence.

11/3,AB/26 (Item 26 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10847005 97198307 PMID: 9046361
Cytokine level changes in L-arginine-induced acute *pancreatitis* in rat.
Takacs T; Czako L; Jarmay K; Farkas G; Mandi Y; Lonovics J First Department of Medicine, Szent-Gyorgyi Albert Medical University, Szeged, Hungary.
Acta physiologica Hungarica (HUNGARY) 1996, 84 (2) p147-56, ISSN 0231-424X Journal Code: 8309201
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The role of different cytokines in the pathogenesis of L-arginine (Arg)-induced acute *pancreatitis* in rat, and the ability of KSG-504, a novel cholecystokinin receptor antagonist, to exert protection in this type of acute *pancreatitis* was evaluated. Male Wistar rats received 250 mg/100 g body weight of Arg intraperitoneally twice, at an interval of 1 h. Control rats received instead the same amount of glycine at the same times. Fifty mg/kg KSG-504 was injected subcutaneously 0.5 h before and 6, 18 and 36 h after the first Arg administration. Rats were examined 12, 24 and 48 h after *pancreatitis* induction. To assess the severity of inflammation, the edema was quantified, the serum amylase level was measured, and histologic examinations were performed. Serum tumor necrosis factor-alpha (TNF-alpha) and interleukin-6 (*IL*-*6*) levels were determined by bioassay, using the TNF-sensitive WEHI 164 and the *IL*- *6*-dependent B9 cell lines, respectively. In Arg-induced acute *pancreatitis* , the amylase level was increased significantly at 12 h (48.600 +/- 3.980 U/l) and 24 h (30.800 +/- 3.813 U/l) vs. the control group (6.382 +/- 184 U/l). No significant alteration in the ratio pancreatic weight/body weight was found in the different groups. However, in Arg-induced acute *pancreatitis*, both the TNF-alpha (15.1 +/- 6.9 U/ml) and the *IL*-*6* (39.6 +/- 19.2 pg/ml) levels were already elevated significantly at 12 h vs. the controls (3.1 +/- 0.8 U/ml and 15.2 +/- 3.1 pg/ml, respectively) and remained elevated at 24 and 48 h. Simultaneous KSG-504 administration did not modify the measured cytokine levels. No significant changes in plasma CCK levels were observed. In Arg-induced acute *pancreatitis* , histological evaluation revealed diffuse but microfocal necrobiotic alterations. No marked protective effects of KSG-504 were observed on histological sections. These results suggest that excessive doses of Arg induce severe acute *pancreatitis* in rat, with a simultaneous cytokine level elevation. Endogenous CCK does not seem to play an essential role in the pathogenesis of Arg-induced acute *pancreatitis*.

11/3,AB/27 (Item 27 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10785700 97135963 PMID: 8981500
Interleukin-6 is a useful marker for early prediction of the severity of acute *pancreatitis*.
Inagaki T; Hoshino M; Hayakawa T; Ohara H; Yamada T; Yamada H; Iida M; Nakazawa T; Ogasawara T; Uchida

A; Hasegawa C; Miyaji M; Takeuchi T First Department of Internal Medicine, Nagoya City University Medical School, Japan.
Pancreas (UNITED STATES) Jan 1997, 14 (1) p1-8, ISSN 0885-3177 Journal Code: 8608542
Comment in Pancreas. 1998 May;16(4) 557-8; Comment in PMID 9598821 Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Twelve patients with acute *pancreatitis* admitted to our department between January 1993 and December 1994 were studied prospectively and classified into two groups (severe group, five patients; mild group, seven patients), according to the criteria for grading severity of acute *pancreatitis* proposed by the Research Committee for Intractable Diseases of the Pancreas, Japanese Ministry of Health and Welfare (1990). To evaluate markers for early estimation of the severity of acute *pancreatitis*, we measured serum changes in various parameters. In the severe group interleukin-6 (*IL*-*6*) levels were increased significantly 5, 24, 72, and 120 h after the onset ($p < 0.01$), compared with the mild group. C-reactive protein (CRP), thrombin antithrombin III, and alpha 2-plasmin inhibitor plasmin complex levels were significantly increased only at the 72-h time point. Peak values of interleukin-8 (IL-8) and soluble human E selectin were observed at 5 and 72 h, respectively, after the onset. There was a significant correlation between *IL*- *6* at 5 h and both pancreatic secretory trypsin inhibitor ($r = 0.85$) and CRP ($r = 0.94$) at 72 h. We therefore conclude that *IL*-*6* is a useful marker for assessment of the severity of acute *pancreatitis* in its early stages.

11/3,AB/28 (Item 28 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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10779474 97129618 PMID: 8974126
Effect of glutamine on immune function in the surgical patient. O'Riordain M G; De Beaux A; Fearon K C
Department of Surgery, Meath Hospital, Ireland, United Kingdom. Nutrition (Burbank, Los Angeles County, Calif.) (UNITED STATES) Nov-Dec 1996, 12 (11-12 Suppl) pS82-4, ISSN 0899-9007 Journal Code: 8802712
Document type: Clinical Trial; Journal Article; Randomized Controlled Trial; Review; Review, Tutorial
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The beneficial effects of glutamine on immune function in vitro have been well described. Severely ill surgical patients undergo glutamine depletion and this has been implicated as a cause of immune dysfunction in vivo. With the introduction of the stable dipeptides of glutamine into total parenteral nutrition (TPN) regimens, the clinical effects of glutamine on the immune system have taken on an increased relevance and importance. In a randomized clinical trial, we have shown that glutamine-supplemented TPN increased the T cell mitogenic response in patients undergoing colorectal resection. This was not associated with an altered production of the pro-inflammatory cytokines interleukin-6 (*IL*-*6*) or tumor necrosis factor (TNF). In a subsequent clinical trial comparing glutamine-supplemented TPN with control TPN in patients with severe acute *pancreatitis* there was a similar modest enhancement of the T cell response in the glutamine-supplemented group. Although *Il*-*6* and TNF production were again unchanged, there was a significant reduction in IL-8 production in the glutamine-supplemented group. Glutamine may exert its immunological effects by a direct action on the cells of the immune system. Possible indirect mechanisms by which glutamine may influence the immune system include the maintenance of gut barrier function, or the preservation of action of the antioxidant glutathione.

11/3,AB/29 (Item 29 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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10704721 97054047 PMID: 8898426
Neopterin in acute *pancreatitis*.
Uomo G; Spada O A; Manes G; Feola B; Misso S; Cavallera A; Rabitti P G Dept. of Gastroenterology, Cardarelli Hospital, Naples, Italy. Scandinavian journal of gastroenterology (NORWAY) Oct 1996, 31 (10) p1032-6, ISSN 0036-5521 Journal Code: 0060105
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
BACKGROUND: Activation of the cellular immune system may play a role in the pathogenesis of acute *pancreatitis* (AP); it has recently been proposed that excessive leukocyte stimulation may lead to the most severe forms of AP. The aim of this study was to investigate serum neopterin, a useful in vivo marker of macrophage activation, in mild and severe AP and its relationship with other markers of leukocyte activation, such as interleukin-6 (*IL*-*6*) and tumor necrosis factor (TNF). METHODS: Serum levels of neopterin (mmol/ml), *IL*-*6* (pg/ml), and TNF (pg/ml) were measured on the 1st and 7th day of hospitalization in 17 patients with severe AP and 24 with mild AP. Severe AP was defined in accordance with the Atlanta criteria: all patients have necrosis at

contrast-enhanced computerized tomography scan. RESULTS: Day 1: Neopterin and *IL*-*6* levels were significantly higher in severe than in mild AP and normal controls; mild AP values were also significantly higher than in normal controls. The best neopterin cutoff level we obtained (30 mmol/ml) reached a specificity of 76% and a sensitivity of 46% in distinguishing severe from mild AP. Day 7: Neopterin was significantly higher in severe AP than in mild AP and in normal controls; no difference was seen between mild AP values and normal controls; neopterin serum levels were significantly higher on day 7 than on day 1 in severe AP but not in mild AP; in both groups of patients *IL*-*6* was significantly higher on day 1 than on day 7. Using a neopterin cutoff level of 40 mmol/ml, we found specificity and sensitivity value of 92% in differentiating severe from mild AP. With regard to TNF values, no difference was seen on day 1 and 7 in the two groups of patients in comparison with normal controls. Neopterin serum values did not correlate with *IL*-*6* and TNF on either day. CONCLUSIONS: These results confirm the activation of the cellular immune system in AP. Initially enhanced NEOP and *IL*-*6* serum levels reflect the severity of the disease; neopterin may be considered a reliable prognostic indicator also at a distance from AP onset because its levels increase during the 1st week of AP in patients with severe forms only.

11/3,AB/30 (Item 30 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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10692549 97041767 PMID: 8887038
Effects of high-dose intraperitoneal aprotinin treatment on complement activation and acute phase response in acute severe *pancreatitis*. Berling R; Ohlsson K
Department of Anaesthesiology, University Hospital MAS, Malmo, Sweden. Journal of gastroenterology (JAPAN) Oct 1996, 31 (5) p702-9, ISSN 0944-1174 Journal Code: 9430794
Document type: Clinical Trial; Journal Article; Multicenter Study; Randomized Controlled Trial
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Forty-eight patients with severe acute *pancreatitis* were treated with intraperitoneal lavage in a double-blind randomized multi-center trial. One group (aprotinin group, n = 22) was also treated intraperitoneally with high doses of the protease inhibitor aprotinin. In the group not treated with aprotinin (control group), 6 patients were operated on because of pancreatic necrosis, compared with none in the treated group. Complement activation and the acute phase response were studied with measurements of anaphylatoxin C3a, C1 inhibitor (C1 Inh), interleukin 6 (*IL*-*6*), and C-reactive protein (CRP). The control group had higher plasma levels of C3a and lower levels of C1 Inh compared with the aprotinin group. The differences were statistically significant for C3a but not for C1 Inh. Both groups had high plasma levels of *IL*-*6* and CRP. There were no differences between the groups in CRP levels, but the control group had higher *IL*-*6* levels (not statistically significant) than the aprotinin group. This was caused by very high levels in the 6 patients operated on because of pancreatic necrosis, indicating that *IL*-*6* could be a good plasma marker of pancreatic necrosis. The results also show that massive antiprotease treatment reduces complement activation, as illustrated by the lower C3a levels in the aprotinin group. The lower C1 Inh levels in the control group could have been caused by an increased consumption of the inhibitor.

11/3,AB/31 (Item 31 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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10680540 97029691 PMID: 8875701
Time-course changes in serum cytokine levels in two experimental acute *pancreatitis* models in rats.
Takacs T; Farkas G; Czako L; Jarmay K; Mandi Y; Lonovics J First Department of Medicine, Albert Szent-Gyorgyi Medical University, Szeged, Hungary.
Research in experimental medicine. Zeitschrift fur die gesamte experimentelle Medizin einschliesslich experimenteller Chirurgie (GERMANY) 1996, 196 (3) p153-61, ISSN 0300-9130 Journal Code: 0324736
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Activated leukocytes and cytokines have important roles in the multi-system involvement during acute *pancreatitis*. The changes in the serum level of tumor necrosis factor-a (TNF-alpha) and interleukin-6 (*IL*-*6*) over time were investigated in two experimental acute *pancreatitis* models in rats. Mild edematous *pancreatitis* was induced with an overdose of cholecystokinin octapeptide (CCK-8), while a severe hemorrhagic form of *pancreatitis* was induced by ligation of the common bilio-pancreatic duct. The rats were examined 2, 4, 8, 16, 24 and 48 h after *pancreatitis* induction. The severity of the inflammation was assessed by measurement of the serum amylase activity, quantification of the edema, and histological examination. Serum TNF-alpha and *IL*-*6* were determined by bioassay, using the TNF-sensitive WEHI 164 and the *IL*-*6*-dependent B9 cell lines,

respectively. In CCK-8-induced acute *pancreatitis*, the pancreatic weight/body weight ratio (pw/bw) and amylase level were significantly elevated at 2 h, and the maximum levels were observed at 4 h (8.19 +/- 1.13 mg/g and 69.4 +/- 12.8 x 10(3) U/ml, respectively). Both parameters subsequently decreased continuously during the observation period. The serum *IL*-*6* level was significantly increased at 4 h relative to the controls (123.3 +/- 5.8 vs 37.5 +/- 15 pg/ml), and then decreased continuously. In this model, only a moderate level of serum TNF-alpha was observed at 2 h. In the biliary type of acute *pancreatitis* , the ratio pw/bw increased continuously during the study and reached the maximum level at 48 h relative to the sham-operated control (8.8 +/- 1.4 vs 5.3 +/- 0.8 mg/g). The serum amylase level was significantly elevated at 2 h (43.2 +/- 13 x 10(3) U/ml), but then decreased continuously. The serum *IL*-*6* reached its maximum level at 16 h (3800 +/- 447 pg/ml). In this model, increased TNF-alpha levels (75-300 U/ml) were measured 8, 16 and 24 h after *pancreatitis* induction. The results led to correlations between the serum *IL*-*6* levels and the biochemical and morphological severity of acute *pancreatitis* in both experimental models. The data suggest that *IL*-*6* and TNF-alpha may participate in the pathogenesis of these types of acute *pancreatitis*.

11/3,AB/32 (Item 32 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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10673858 97022945 PMID: 8869305
Proinflammatory cytokine release by peripheral blood mononuclear cells from patients with acute *pancreatitis*.
de Beaux A C; Ross J A; Maingay J P; Fearon K C; Carter D C University Department of Surgery, Royal Infirmary, Edinburgh, UK. British journal of surgery (ENGLAND) Aug 1996, 83 (8) p1071-5, ISSN 0007-1323 Journal Code: 0372553
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Proinflammatory cytokine release was measured from peripheral blood mononuclear cells (PBMCs) isolated from six volunteers and, on admission, from 16 patients with acute *pancreatitis*. Tumour necrosis factor (TNF) release in patients did not differ significantly from that of volunteers, whereas both interleukin (*IL*) *6* and IL-8 release in patients was raised when compared with that in the volunteer group (mean(s.e.m.) *IL*-*6* 20.7(4.6) versus 9.3(1.7) ng/ml, P = 0.03; IL-8 283(40) versus 128(22) ng/ml, P = 0.04). When variation in white cell count was accounted for, *IL*-*6* and IL-8 release but not that of TNF was significantly greater in patients with severe disease than in those with mild disease. These results point to a complex upregulation of proinflammatory cytokine release from PBMCs in patients with acute *pancreatitis* , components of which relate to the clinical progress of the disease.

11/3,AB/33 (Item 33 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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10593713 96408790 PMID: 8813775
Increased monocyte cytokine production in association with systemic complications in acute *pancreatitis*.
McKay C J; Gallagher G; Brooks B; Imrie C W; Baxter J N
University Department of Surgery, Glasgow Royal Infirmary, UK. British journal of surgery (ENGLAND) Jul 1996, 83 (7) p919-23, ISSN 0007-1323 Journal Code: 0372553
Document type: Clinical Trial; Journal Article; Randomized Controlled Trial
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Tumour necrosis factor (TNF) alpha, interleukin (IL) 1 beta, *IL*- *6* and IL-8 are thought to play a central role in the pathophysiology of sepsis but their role in acute *pancreatitis* is unknown. In the present study, monocytes were isolated from the peripheral blood of 26 patients with moderate or severe acute *pancreatitis* without biliary sepsis. Secretion of these cytokines in vitro was measured at intervals during the first week of illness. Sixteen patients developed systemic complications. Peak TNF-alpha secretion was significantly higher in patients who developed systemic complications (median (interquartile range (i.q.r.)) 18.5 (5.5-28.5) ng/ml) than in those with an uncomplicated course (3.7 (2.3-6.4) ng/ml, P < 0.01). Similarly, peak *IL*-*6* and peak IL-8 secretion were significantly higher in the complicated group (*IL* -*6*: complicated median (i.q.r.) 48.9 (12.1-71.0) ng/ml, uncomplicated 16.3 (14.2-37.9) ng/ml, P < 0.05; IL-8: complicated 748 (643-901) ng/ml, uncomplicated 608 (496-749) ng/ml), P < 0.05). No significant difference in peak IL-1 beta secretion was observed between the two groups. Systemic complications of acute *pancreatitis* are associated with a significant increase in monocyte secretion of TNF-alpha, *IL*-*6* and IL-8 suggesting that, as in sepsis, these cytokines play a central role in the pathophysiology of the disease.

11/3,AB/34 (Item 34 from file: 155)

DIALOG(R)File 155:MEDLINE(R)
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10590197 96405100 PMID: 8809240
Extraintestinal considerations in inflammatory bowel disease. Levine J B; Lukawski-Trubish D
University of Connecticut School of Medicine, Farmington, USA. Gastroenterology clinics of North America (UNITED STATES) Sep 1995, 24 (3) p633-46, ISSN 0889-8553 Journal Code: 8706257
Document type: Journal Article; Review; Review, Tutorial
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
If one reviews the literature with zeal, it is increasingly apparent that few organs escape recruitment when IBD is chronic or progressive. Insights into mucosal pathophysiology have helped with understanding the more frequent extraintestinal manifestations, but the mechanisms attendant to the development of less common events (e.g. acute *pancreatitis*, concurrent gluten sensitive enteropathy, or active pulmonary disease) remain either poorly studied or obscure. It is particularly interesting, however, to read reports of abnormal pulmonary function, generally of the obstructive type, correlated to measurements of abnormal intestinal permeability in patients with either active pulmonary sarcoid or pulmonary involvement in Crohn's disease. It has been further speculated that similarities in the mucosal immune system of the lung and intestine are responsible for evidence of bronchial hyperreactivity in patients with active IBD. Finally, it is important to recognize that extensions of the inflammatory process are not restricted to the development of organ-based events but may be responsible for some of the most frequent systemic abnormalities detected in IBD patients. It is now also well confirmed that the cytokine environment in IBD can support activated coagulation and, in some clinical situations, overt vascular thrombosis. The cerebrovascular complications of IBD are well recognized and range from peripheral venous thrombosis to central stroke syndromes and pseudotumor cerebri. Reports of focal white matter lesions in the brains of patients with IBD or an increased incidence of polyneuropathy may be other clinical examples of regional microvascular clotting. Microvascular injury appears to be more ubiquitously present, with reports ranging from a speculated primary causative role (e.g., granulomatous vasculitis in the mesenteric circulation) to the utility of nailbed vasospasm, in Crohn's disease, as a clinical marker for disease activity. It is also reported that *IL*- *6* suppression of erythropoietin production is a major feature of the chronic anemia seen in active IBD. Moreover, the capacity of peripheral monocytes from active IBD patients to secrete TNF and IL-8 is reported predictive for the degree of therapeutic response from recombinant erythropoietin. These collected observations constitute another excellent example of the symmetry between basic science and clinical utility. It is from the context of applied basic science that many future therapies will arise. Empiricism will lose much of its appeal as clinical observations will be increasingly translated into cellular language. Already in animal models, elemental diets diminish *IL*-*6* -related acute inflammatory injury, and reductions in dietary lipid alter the antigenicity of bacteria. Provocatively, in humans, unconfirmed reports have even associated diet therapy with the resolution of uveitis and pyoderma gangrenosum. It is likely that efforts will also be made to induce oral tolerance if specific triggering proteins are discovered or to alter bowel flora if such an arcane area of investigation becomes resurgent.

11/3,AB/35 (Item 35 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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10453365 96260099 PMID: 8661228
Intrapancreatic interleukin-1beta gene expression by specific leukocyte populations during acute *pancreatitis*.
Fink G W; Norman J G
Department of Surgery, University of South Florida, Tampa 33612, USA. Journal of surgical research (UNITED STATES) Jun 1996, 63 (1) p369-73, ISSN 0022-4804 Journal Code: 0376340
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The importance of interleukin-1beta (IL-1beta) in the pathogenesis of acute *pancreatitis* has been demonstrated by dramatic attenuation of pancreatic destruction and significant increases in survival when its actions are inhibited. The pancreas has been shown to be a major producer of IL-1beta during *pancreatitis* but the cell(s) of origin remains unknown. Hypothesizing that infiltrating leukocytes contribute substantially, the intrapancreatic production of IL-1beta was examined after specific leukocyte populations were manipulated in vivo prior to the induction of *pancreatitis* . Sixty-four adult male Swiss mice were assigned to one of four groups 48 hr prior to induction of *pancreatitis* : (1) PMN depletion via anti-murine PMN antiserum. [PMN-d], (2) macrophage (Mphi) depletion via anti-macrophage antiserum [Mphi-d], (3) PMN and Mphi depletion [PMN+Mphi-d], and (4) Immunocompetent *Pancreatitis*. Edematous *pancreatitis* was induced in all experimental groups by caerulein (50 microg/kg/hr ip X 4). Animals were sacrificed 6 hr after induction of *pancreatitis* with

severity determined by blind histologic grading and serum amylase, lipase, and interleukin-6 (*IL*-*6*) levels. Intrapancreatic IL-1beta production was determined by immunohistochemistry and semiquantitative differential RT-PCR. *Pancreatitis* developed in all animals receiving caerulein; however, leukocyte-depleted animals showed significantly attenuated levels of serum amylase, lipase, and *IL*-*6*, as well as lower histologic severity scores. Similarly, *pancreatitis* induction in immunocompetent mice showed pancreatic infiltration of IL-1beta-producing cells, whereas the leukocyte-depleted animals had significantly decreased numbers (PMN+Mphi-d < Mphi-d < PMN-d). IL-1beta mRNA was upregulated in all animals developing *pancreatitis* with significantly lower levels seen in the leukocyte-depleted groups. We conclude that infiltrating leukocytes, both neutrophils and macrophages, are responsible for the majority of intrapancreatic IL-1beta production during acute *pancreatitis* . The elimination of leukocytes and their products, including IL-1beta, significantly decreases the severity of pancreatic destruction.

11/3,AB/36 (Item 36 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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10439583 96246258 PMID: 8665189
Serum concentrations of inflammatory mediators related to organ failure in patients with acute *pancreatitis*.
de Beaux A C; Goldie A S; Ross J A; Carter D C; Fearon K C Department of Surgery, University of Edinburgh, UK.
British journal of surgery (ENGLAND) Mar 1996, 83 (3) p349-53, ISSN 0007-1323 Journal Code: 0372553
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Leucocyte activation and proinflammatory cytokine release (tumour necrosis factor (TNF) and interleukin 6 (*IL*-*6*)) are thought to contribute to the induction of a systemic inflammatory response, an acute-phase response and multiple organ failure in patients with acute *pancreatitis* . The serum concentration of TNF, soluble TNF receptors (sTNFR55 and sTNFR75), *IL*-*6* and C-reative protein (CRP) in 58 patients with acute *pancreatitis* was assessed during the first 2 days of admission. Thirty patients had mild disease and 28 severe disease, of whom 18 developed local pancreatic complications alone (Atlanta classification) and ten developed organ failure (a Goris score of 1 or more). TNF was detected in only 17 patients on the first day of admission, while soluble TNF receptors were detected in all patients and *IL*- *6* in 34. On the first and second days of admission there was a progressive and significant (P < 0.03) increase in the median concentration of sTNFR55, sTNFR75 and *IL*-*6* in patients eventually classified into those with mild disease, a local pancreatic complication alone, or organ failure. This pattern was also evident in CRP levels from the second but not the first day of admission. These findings suggest that proinflammatory cytokines or their soluble receptors may be more accurate early predictors of outcome than CRP, Moreover, markers of inflammation in the sera of patients with acute *pancreatitis* are highest in those who subsequently develop organ failure.

11/3,AB/37 (Item 37 from file: 155)
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10423843 96230249 PMID: 8651687
Induction of lithostathine/reg mRNA expression by serum from rats with acute *pancreatitis* and cytokines in pancreatic acinar AR-42J cells. Dusetti N J; Mallo G V; Ortiz E M; Keim V; Dagorn J C; Iovanna J L U.315 INSERM, Marseille, France.
Archives of biochemistry and biophysics (UNITED STATES) Jun 1 1996, 330 (1) p129-32, ISSN 0003-9861 Journal Code: 0372430 Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
During the acute phase of *pancreatitis* , expression of most pancreatic enzymes decreases, whereas mRNAs of *pancreatitis* associated protein and lithostathine/reg increase dramatically. In the present study we have investigated the effect of serum from rats with acute *pancreatitis* (SAP) and cytokines on the lithostathine/reg mRNA expression in AR-42J cells. Lithostathine/reg mRNA was strongly induced by SAP in a dose-dependent manner. Induction was abolished by preheating the SAP or by treating the cells with cycloheximide. Treatment with interleukins (IL) IL-1 or *IL*-*6* or dexamethasone alone was ineffective. Combination of IL-1 with *IL*-*6* was also ineffective. Combination of *IL*-*6* with dexamethasone resulted in strong induction of the lithostathine/reg gene, but the further addition of IL-1 to the mixture reduced induction. Treatment with tumor necrosis factor-alpha (TNFalpha) or interferon-gamma (IFNgamma) induced lithostathine/reg mRNA expression. Combination of dexamethasone with TNFalpha or IFNgamma showed an inhibitory effect on lithostathine/reg mRNA expression. These findings suggest that expression of the

lithostathine/reg mRNA during acute *pancreatitis* could be mediated by specific combinations of cytokines and/or glucocorticoids.

11/3,AB/38 (Item 38 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10370425 96175365 PMID: 8597510
Active interleukin-1 receptor required for maximal progression of acute *pancreatitis*.
Norman J G; Fink G; Franz M; Guffey J; Carter G; Davison B; Sexton C; Glaccum M
Department of Surgery, University of South Florida, Tampa, Florida, USA.
Annals of surgery (UNITED STATES) Feb 1996, 223 (2) p163-9, ISSN 0003-4932 Journal Code: 0372354
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
OBJECTIVE: The authors' aim was to determine the requirement for an active interleukin (IL)-1 receptor during the development and progression of acute *pancreatitis*. SUMMARY OF BACKGROUND DATA: Interleukin-1 is a pro- inflammatory cytokine that has been shown to be produced during acute *pancreatitis*. Earlier animal studies of moderate and severe *pancreatitis* have shown that blockade of this powerful mediator is associated with attenuated pancreatic destruction and dramatic increases in survival. The exact role played by IL-1 and the requirement for activation of its receptor in the initiation and progression of *pancreatitis* is unknown. METHODS: Conventional and IL-1 receptor knockout animals were used in parallel experiments of acute *pancreatitis* induced by intraperitoneal injection of cerulean (50 microg/kg every 1 hour X 4). The conventional mouse strain had the IL-1 receptor blocked prophylactically by means of a recombinant IL-1 receptor antagonist (10 mg/kg injected intraperitoneally every 2 hours). The second mouse strain was genetically engineered by means of gene targeting in murine embryonic stem cells to be devoid of type 1 IL-1 receptor (IL-1 receptor knockout). Animals were killed at 0, 0.5, 1, 2, 4, and 8 hours, with the severity of *pancreatitis* determined by serum amylase, lipase, and *IL*- *6* levels and blind histologic grading. Strain-specific controls were used for comparison. RESULTS: The genetic absence of the IL-1 receptor or its pharmacologic blockade resulted in significantly attenuated pancreatic vacuolization, edema, necrosis, inflammation, and enzyme release. Serum *IL*-*6* , a marker of inflammation severity, was dramatically decreased in both groups. CONCLUSIONS: Activation of the IL-1 receptor is not required for the development of *pancreatitis* but apparently is necessary for the maximal propagation of pancreatic injury and its associated inflammation.

11/3,AB/39 (Item 39 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10295882 96097842 PMID: 7489182
Randomized, double-blind phase II trial of Lexipafant, a platelet-activating factor antagonist, in human acute *pancreatitis*.
Kingsnorth A N; Galloway S W; Formela L J
Department of Surgery, University of Liverpool, UK.
British journal of surgery (ENGLAND) Oct 1995, 82 (10) p1414-20, ISSN 0007-1323 Journal Code: 0372553
Document type: Clinical Trial; Clinical Trial, Phase II; Journal Article; Randomized Controlled Trial
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The aims of the study were to determine whether the platelet-activating factor antagonist Lexipafant could alter the clinical course and suppress the inflammatory response of human acute *pancreatitis* . In a double-blind, placebo-controlled study 83 patients were randomized to receive Lexipafant 60 mg intravenously for 3 days, or placebo. Clinical progression was assessed by daily Acute Physiology And Chronic Health Evaluation (APACHE) II score and organ failure score (OFS). The magnitude of the inflammatory response on days 1-5 was assessed by serial measurement of interleukin (IL) 8, *IL*-*6* , E-selectin, polymorphonuclear elastase-alpha1-antitrypsin (PMNE-alpha 1-AT), and C-reactive protein (CRP). At entry, patients receiving Lexipafant (n = 42) or placebo (n = 41) were matched for age and sex, aetiology, APACHE II score and OFS. The disease was classified as severe in 29 patients (APACHE II score eight or more). There was a significant reduction in the incidence of organ failure (P = 0.041) and in total OFS (P = 0.048) at the end of medication (72 h). During this time seven of 12 patients with severe acute *pancreatitis* who had Lexipafant recovered from an organ failure; only two of 11 with severe acute *pancreatitis* who had placebo recovered from an organ failure and two others developed new organ failure. Lexipafant treatment significantly reduced serum IL-8 (P = 0.038), and *IL*-*6* declined on day 1. Plasma PMNE-alpha 1-AT complexes peaked on day 1; the gradual fall to baseline over 5 days observed in controls did not occur in patients given Lexipafant. No effect was observed on serum CRP. This study provides a rationale for further clinical trials with the potent PAF antagonist Lexipafant in human

acute *pancreatitis*.

11/3,AB/40 (Item 40 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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10247392 96048808 PMID: 7582288
Lymphatic release of cytokines during acute lung injury complicating severe *pancreatitis*.
Montravers P; Chollet-Martin S; Marmuse J P; Gougerot-Pocidalo M A; Desmonts J M
Departement d'Anesthesie Reanimation Chirurgicale, Hopital Bichat, Paris, France.
American journal of respiratory and critical care medicine (UNITED STATES) Nov 1995, 152 (5 Pt 1) p1527-33, ISSN 1073-449X Journal Code: 9421642
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
In severe acute *pancreatitis* (SAP), the mechanisms leading to adult respiratory distress syndrome (ARDS) are usually attributed to the release of active enzymes and vasoactive substances from the pancreas. Thoracic duct drainage has been proposed as a means of removing the portion of these substances that drain through retroperitoneal lymphatics before they reach the systemic circulation. This technique was used in six patients with ARDS complicating SAP. The levels of proinflammatory cytokines (tumor necrosis factor-alpha [TNF alpha], interleukin-1 [IL-1], and interleukin-6 [*IL* -*6*]), neutrophil enzymes (myeloperoxidase and lactoferrin), and pancreatic enzymes (amylase, lipase and trypsin) were measured in plasma and lymph in the first 24 h of ARDS and then on Day 2, Day 4, and at the end of the drainage (Day 8). High plasma concentrations of these products were measured. A moderate lymph-to-plasma gradient was observed for *IL*-*6* , lipase, and trypsin, while similar levels in plasma and lymph were recorded for the other substances. Plasma levels of pancreatic enzymes were weakly correlated with the lung injury score and lymph level of cytokines. These results suggest that in patients with ARDS due to SAP, cytokines as well as pancreatic enzymes could contribute to the development of the lung injury, and that lymphatics are potential vectors of these mediators.

11/3,AB/41 (Item 41 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08714836 95403441 PMID: 7545677
Pancreatitis -associated protein I (PAP I), an acute phase protein induced by cytokines. Identification of two functional interleukin-6 response elements in the rat PAP I promoter region.
Dusetti N J; Ortiz E M; Mallo G V; Dagorn J C; Iovanna J L Unite 315, INSERM, Marseille, France.
Journal of biological chemistry (UNITED STATES) Sep 22 1995, 270 (38) p22417-21, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Expression of the *pancreatitis* -associated protein I (PAP I), an exocrine pancreatic protein, increases rapidly and strongly in acinar cells during the acute phase of *pancreatitis*. This is reminiscent of the response to stress of acute phase proteins. We have previously demonstrated that serum factors from rats with acute *pancreatitis*, but not from healthy rats, could induce endogenous PAP I gene expression in the acinar cell line AR-42J (Dusetti, N., Mallo, G., Dagorn, J.-C., Iovanna, J. L. (1994) Biochem. Biophys. Res. Commun. 204, 238-243). In the present work, we have evaluated the influence of several mediators of inflammation on rat PAP I gene transcription in these cells. Tumor necrosis factor alpha induced an increase in PAP I mRNA expression, and interferon gamma caused an even greater increase in PAP I mRNA level. These stimulations were antagonized by dexamethasone. Interleukin (IL)-1, *IL*-*6*, or dexamethasone alone were ineffective. Combinations of IL-1 with *IL*- *6* or dexamethasone were also ineffective. *IL*-*6* and dexamethasone together induced a marked stimulation of PAP I gene transcription, and this effect was slightly attenuated by IL-1. To analyze the cis-regulatory elements responsible for the induction of transcription, we fused a 1.2-kilobase segment of the rat PAP I promoter to the chloramphenicol acetyltransferase (CAT) gene as reporter. The resultant chimeric DNA was transfected into AR-42J cells. Addition of *IL*- *6* or dexamethasone was ineffective, whereas their mixture increased the CAT activity 12 times. Progressive deletions of the PAP I promoter were then fused to the CAT gene, and the constructs were transfected to AR-42J cells. A 12-fold increase in CAT activity was seen upon *IL*-*6* /dexamethasone treatment with constructs containing more than 274 base pairs upstream from the cap site. In that region, two sequences are similar to the canonical *IL*-*6* response element. Site-directed mutagenesis of these regions strongly decreased induction, showing that they were functional. PAP I should therefore be classified among acute phase proteins of class 2, whose expression is increased by *IL*- *6* acting in combination with glucocorticoids.

11/3,AB/42 (Item 42 from file: 155)

DIALOG(R)File 155:MEDLINE(R)
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08691554 95380159 PMID: 7651717
Inflammatory mediators in acute *pancreatitis* (theoretical considerations)]
Gyulladaskelto mediatorok heveny hasnyalmirigy-gyulladasban (elmeleti megfontolasok).
Farkas G
Szent-Gyorgyi Albert Orvostudomanyi Egyetem Sebeszeti Klinika, Szeged. Orvosi hetilap (HUNGARY) Aug 20 1995, 136 (34) p1819-22, ISSN 0030-6002 Journal Code: 0376412
Document type: Journal Article; Review; Review Literature ; English Abstract
Languages: HUNGARIAN
Main Citation Owner: NLM
Record type: Completed
Cytokines are important immunoregulatory mediators. Their contribution to the pathogenesis of acute and chronic gastroenterological disorders is obvious. Increased expression of interleukin-1 (IL-1), interleukin-6 (*IL*-*6*) and tumor necrosis factor (TNF) can be detected in inflammatory bowel disease. During the last few years it has also been recognized that activated leukocytes have an important role in the multisystem involvement of acute *pancreatitis* . Activation of leukocytes is an early event during severe acute *pancreatitis*, and it may be a pathogenetic factor in the severity of the disease. The review summarizes the recent findings in the field of inflammatory cytokines with particular attention of TNF, IL-1, *IL*-*6*, and IL-8 during severe acute *pancreatitis* and underscores the role of the activated leukocytes in the pathogenesis of complicated acute *pancreatitis*.

11/3,AB/43 (Item 43 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08625884 95314411 PMID: 7794067
Decreased mortality of severe acute *pancreatitis* after proximal cytokine blockade.
Norman J G; Franz M G; Fink G S; Messina J; Fabri P J; Gower W R; Carey L C
Department of Surgery, University of South Florida, Tampa, USA. Annals of surgery (UNITED STATES) Jun 1995, 221 (6) p625-31; discussion 631-4, ISSN 0003-4932 Journal Code: 0372354
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
OBJECTIVE: This study determined the ability of interleukin-1 receptor antagonist (IL-1ra) to decrease the mortality of experimental acute *pancreatitis* . The response of the inflammatory cytokine cascade and its subsequent effects on pancreatic morphology were measured to determine the role of these peptides in mediating pancreatic injury. SUMMARY BACKGROUND DATA: Previous studies have shown that proinflammatory cytokines are produced in large amounts during acute *pancreatitis* and that blockade at the level of the IL-1 receptor significantly decreases intrinsic pancreatic damage. The subsequent effect on survival is not known. METHODS: A lethal form of acute hemorrhagic necrotizing *pancreatitis* was induced in young female mice by feeding a choline-deficient, ethionine supplemented (CDE) diet for 72 hours. For determination of mortality, the animals were divided into 3 groups of 45 animals each: control subjects received 100/microL normal saline intraperitoneally every 6 hours for 5 days; IL-1ra early mice received recombinant interleukin-1 receptor antagonist 15 mg/kg intraperitoneally every 6 hours for 5 days beginning at time 0; IL-1ra late mice received IL-1ra 15 mg/kg intraperitoneally every 6 hours for 3.5 days beginning 1.5 days after introduction of the CDE diet. A parallel experiment was conducted simultaneously with a minimum of 29 animals per group, which were sacrificed daily for comparisons of serum amylase, lipase, IL-1, *IL*- *6* , tumor necrosis factor-alpha, IL-1ra, pancreatic wet weight, and blind histopathologic grading. RESULTS: The 10-day mortality in the untreated control group was 73%. Early and late IL-1ra administration resulted in decreases of mortality to 44% and 51%, respectively (both $p < 0.001$). Interleukin-1 antagonism also was associated with a significant attenuation in the rise in pancreatic wet weight and serum amylase and lipase in both early and late IL-1ra groups (all $p < 0.05$). All control animals developed a rapid elevation of the inflammatory cytokines, with maximal levels reached on day 3. The IL-1ra-treated animals, however, demonstrated a blunted rise of these mediators (all $p < 0.05$). Blind histologic grading revealed an overall decrease in the severity of *pancreatitis* in those animals receiving the antagonist. CONCLUSIONS: Early or late blockade of the cytokine cascade at the level of the IL-1 receptor significantly decreases the mortality of severe acute *pancreatitis* . The mechanism by which this is accomplished appears to include attenuation of systemic inflammatory cytokines and decreased pancreatic destruction.

11/3,AB/44 (Item 44 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08624012 95312539 PMID: 7540760
The relationship between pancreatic enzyme release and

activation and the acute-phase protein response in patients with acute *pancreatitis*. Heath D I; Cruickshank A; Gudgeon A M; Jehanli A; Shenkin A; Imrie C W Department of Surgery, Glasgow Royal Infirmary, Scotland. Pancreas (UNITED STATES) May 1995, 10 (4) p347-53, ISSN 0885-3177 Journal Code: 8608542
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
It has been suggested that the severity of an attack of acute *pancreatitis* is related to the presence of intraglandular trypsinogen activation and that disease severity is also reflected by the degree of the acute-phase protein response. In this study we examine the relationships among amylase release, the degree of trypsinogen and prophospholipase A2 activation [as measured by urinary trypsinogen activation peptide (TAP) and prophospholipase A2 activation peptide (PLAP) concentrations], and the serum concentrations of the acute phase-protein C-reactive protein (CRP) and the principal mediator of the acute-phase protein response, interleukin-6 (*IL*-*6*). Twenty-four patients (14 mild and 10 severe attacks) were studied. Peak serum amylase concentrations were seen within 12 h and peak urinary TAP/creatinine (Cr) and PLAP/Cr ratios between 12 and 24 h after the onset of symptoms, preceding those of *IL*- *6* and CRP. The integrated TAP/Cr and PLAP/Cr responses were significantly greater in those with severe disease [95% confidence internal (CI) = 106-259.6 pmol/mmol/h, p < 0.0008; and 95.1% CI = 462.2-3887 pmol/mmol/h, p < 0.003, respectively]. The integrated amylase response was not significantly greater in those with severe disease (95.6% CI = -415 to 832 IU/L/h, p < 0.14). There was a strong correlation among the integrated *IL*-*6* , TAP/Cr (r = 0.63, p < 0.01), and PLAP/Cr (r = 0.64, p < 0.01) responses but a poor correlation with the integrated amylase response (r = 0.19, NS).(ABSTRACT TRUNCATED AT 250 WORDS)

11/3,AB/45 (Item 45 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08608342 95296868 PMID: 7539942
Interleukin-1 receptor antagonist decreases severity of experimental acute *pancreatitis*.
Norman J; Franz M; Messina J; Riker A; Fabri P J; Rosemurgy A S; Gower W R
Department of Surgery, University of South Florida, Tampa 33612, USA. Surgery (UNITED STATES) Jun 1995, 117 (6) p648-55, ISSN 0039-6060 Journal Code: 0417347
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
BACKGROUND. Fulminant acute *pancreatitis* is a disease of complex origin that results in activation of several of the proinflammatory cytokines. Because interleukin-1 (IL-1) is an integral early component of the acute inflammatory process, the use of an IL-1 receptor antagonist (IL-1ra) was investigated in experimental acute *pancreatitis* to determine the therapeutic potential of proximal cytokine blockade and to further establish the role of inflammatory cytokines in the pathogenesis of acute *pancreatitis* . METHODS. IL-1ra was administered in escalating doses either before or after acute edematous, necrotizing *pancreatitis* was induced in adult male mice by injection of cerulein. The severity of *pancreatitis* was quantified by serum amylase, lipase, interleukin-6 (*IL*-*6*), and tumor necrosis factor-alpha (TNF-alpha) levels, pancreatic wet weight, and blinded histologic grading. RESULTS. Administration of medium (10 mg/kg) and high (100 mg/kg) doses of IL-1ra either before or after the induction of *pancreatitis* significantly decreased the expected rise in pancreatic wet weight, lipase, *IL*-*6* , and TNF-alpha (all, p < 0.01). Serum amylase was significantly reduced when IL-1ra was administered in either dosage before (p < 0.05), but not after, induction of *pancreatitis*. *Pancreatic* *edema* , necrosis, and inflammatory cell infiltrate were significantly diminished (p < 0.05) by histologic grading in all animals receiving medium or high doses of IL-1ra. Low doses of IL-1ra (1.0 mg/kg) had modest effects if given before, but no effect if given after, induction of *pancreatitis*. CONCLUSIONS. The proinflammatory cytokines *IL*-*6* and TNF-alpha are elevated during experimental acute *pancreatitis* and correlate well with the severity of local pancreatic destruction. Blockade of the cytokine cascade at the level of the IL-1 receptor before or soon after induction of *pancreatitis* significantly attenuates the rise in these cytokines and is associated with decreased severity of *pancreatitis* and reduced intrinsic pancreatic damage.

11/3,AB/46 (Item 46 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08498387 95186661 PMID: 7533549
Interleukin-6 regulation of CCK/gastrin receptors and amylase secretion in a rat pancreatic acinar cell line (AR4-2J).
Viguerie N; Bertrand V; Dufresne M; Davis E; Lefort S; Vita N; Vaysse N; Pradayrol L; Bastie M J
INSERM U151, Institut Louis Bugnard, C.H.U. Rangueil, Toulouse, France. European cytokine network (FRANCE) Sep-Oct 1994, 5 (5) p433-40, ISSN 1148-5493

Journal Code: 9100879
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
In necrotizing *pancreatitis* a high interleukin-6 (*IL*-*6*) serum level has been proposed as a prognostic criterium. However, literature does not report any information about the role of *IL*- *6* in the function of pancreatic acinar cells. Cholecystokinin, gastrin binding, amylase release and intracellular calcium measurement were performed on a rat pancreatoma cell line, AR4-2J, which has been recognized as a useful tool for studies on the long-term regulation of pancreatic acinar cells. The addition of *IL*-*6* (400 pM) for 48hrs to the AR4-2J cells induced no change in the binding affinities but there was a 2 fold increase in the binding capacity of cholecytokinin (CCKA R) and gastrin (CCKB R) receptors. Although *IL*-*6* treatment did not change directly the secretory capacity and did not activate the intracellular calcium mobilization of AR4-2J, it indirectly increased the sensitivity of the cells to the stimulation of amylase release and the intracellular calcium mobilization by cholecystokinin and gastrin. It is most likely this effect was due to the *IL*-*6*-induced increase in the numbers of CCKA R and CCKB R. Therefore this report suggests that the cytokine *IL*-*6* acts on the CCK regulation of pancreatic enzyme secretion.

11/3,AB/47 (Item 47 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08274000 94340065 PMID: 8061800
Serum levels of interleukin-1 beta and interleukin-6 in patients with chronic *pancreatitis*.
Bamba T; Yoshioka U; Inoue H; Iwasaki Y; Hosoda S
Second Department of Internal Medicine, Shiga University of Medical Science, Japan.
Journal of gastroenterology (JAPAN) Jun 1994, 29 (3) p314-9, ISSN 0944-1174 Journal Code: 9430794
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
To investigate the role played by cytokines in chronic *pancreatitis* , we examined serum levels of interleukin-1 beta (IL-1 beta) and interleukin-6 (*IL*-*6*) by radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA) in 33 patients with definitively diagnosed chronic *pancreatitis* . All the patients, who had received either no treatment or only digestive enzyme products for their chronic *pancreatitis*, had significantly elevated serum IL-1 beta levels (38.5 +/- 28.8 pg/ml, mean +/- SD), compared to normal controls (16.0 +/- 6.7 pg/ml; P < 0.01); however they showed no changes in serum *IL*-*6* levels. Changes in IL-1 beta and *IL*-*6* serum levels were not correlated with the etiological features of *pancreatitis* or with complications due to liver diseases. Serum IL-1 beta and *IL*-*6* levels were also not correlated with the activity of any pancreatic enzymes in blood or urine. However, in the patients with chronic *pancreatitis* , serum *IL*-*6* levels were correlated with C-reactive protein (CRP), whereas serum IL-1 beta levels were not correlated with CRP or with erythrocyte sedimentation rate. These results suggest that serum IL-1 beta is involved in the progression and reduction of chronic inflammation of the pancreas, and that the serum IL-1 beta level may be useful as a marker for chronic *pancreatitis*.

11/3,AB/48 (Item 48 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08237830 94303787 PMID: 8030746
Islet inflammation and hyperplasia induced by the pancreatic islet-specific overexpression of interleukin-6 in transgenic mice. Campbell I L; Hobbs M V; Dockter J; Oldstone M B; Allison J Department of Neuropharmacology, Scripps Research Institute, La Jolla, California 92037.
American journal of pathology (UNITED STATES) Jul 1994, 145 (1) p157-66, ISSN 0002-9440 Journal Code: 0370502
Contract/Grant No.: AG09822; AG; NIA; MH50456; MH; NIMH; NS12428; NS; NINDS; +
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Interleukin-6 (*IL*-*6*) is thought to be involved in the pathogenesis of autoimmune insulin-dependent diabetes mellitus. To examine this possibility, we developed two lines of transgenic mice (termed RIP-IL6) which overexpressed *IL*-*6* in the pancreatic islet beta cells. RIP-IL6 mice, while showing a modest reduction in body weight, remained normoglycemic throughout their lives. Furthermore, insulin gene expression and glucose tolerance were similar to non-transgenic littermates. Histopathological examination revealed significant changes in the pancreas but not other organs of RIP-IL6 animals, with marked alterations in the architecture of the islets, in the islet cells, and in surrounding tissues. In younger animals these changes included islet hyperplasia with increased mitotic figures, neo-ductular formation,

fibrosis, and a scant mononuclear cell infiltration (insulitis). In addition, immunostaining for islet hormones revealed changes in both the topography and density of beta and alpha cells. In older RIP-IL6 mice, a more florid insulitis was observed which was composed predominantly of B220+ B lymphocytes and, to a lesser extent, Mac-1+ macrophages and CD4+ and CD8+ T lymphocytes. Immunostaining for mouse IgG revealed significant numbers of plasma cells in the peri-islet infiltrates, which suggested that *IL*-*6* induced differentiation of the recruited B lymphocytes. Therefore, islet overexpression of *IL*-*6* produces a complex, localized host response implicating this cytokine in not only inflammatory processes that occur in autoimmune diabetes but also cellular neogenesis, which may indicate a role in tissue repair.

11/3,AB/49 (Item 49 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08098830 94164590 PMID: 7509768
Inflammatory mediators and cytokines--new aspects of the pathophysiology and assessment of severity of acute *pancreatitis*?
Gross V; Leser H G; Heinisch A; Scholmerich J
Department of Internal Medicine I, University of Regensburg, Germany. Hepato-gastroenterology (GERMANY) Dec 1993, 40 (6) p522-30, ISSN 0172-6390 Journal Code: 8007849
Document type: Journal Article; Review; Review, Academic Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Most attacks of acute *pancreatitis* are mild and self-limiting. In 10-20% of the cases, however, severe disease with multiple systemic complications develops. During the last few years it has been recognized that activated leukocytes have an important role in the multisystem involvement during acute *pancreatitis*. Activated leukocytes are thus a pathogenetic factor in the severity of the disease. Activation of polymorphonuclear granulocytes (PMNs) and of monocytes/macrophages is an early event during severe acute *pancreatitis*. Factors released by activated leukocytes therefore reflect the severity of the disease. Three independent studies have shown that released PMN-elastase is a reliable early prognostic marker that permits correct classification of 80-95% of the patients within the first 24-48 hours. Interleukin-6 (*IL*-*6*), mainly secreted by activated monocytes/macrophages, is also an early prognostic parameter (shown in one study), but is not superior to PMN-elastase. Leukocyte activation markers are more reliable than multiple scoring systems in the assessment of the severity of acute *pancreatitis*. Compared with PMN-elastase or *IL*-*6* , increased plasma concentrations of such acute-phase proteins as alpha-1-antitrypsin or CRP, and consumption of the protease inhibitor alpha-2-macroglobulin, are later events that can be detected only 1 to 4 days later. Comparison of the various inflammatory parameters suggests that PMN-elastase is the best early and reliable prognostic marker in acute *pancreatitis* . The reviewed data underscore the role of activated leukocytes in the pathogenesis of complicated acute *pancreatitis*.

11/3,AB/50 (Item 50 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07995746 94061463 PMID: 7902182
Reduction in circulating levels of CD4-positive lymphocytes in acute *pancreatitis* : relationship to endotoxin, interleukin 6 and disease severity.
Curley P J; McMahon M J; Lancaster F; Banks R E; Barclay G R; Shefta J; Boylston A W; Whicher J T
Academic Unit of Surgery, General Infirmary, Leeds, UK.
British journal of surgery (ENGLAND) Oct 1993, 80 (10) p1312-5, ISSN 0007-1323 Journal Code: 0372553
Comment in Br J Surg. 1994 Feb;81(2) 312; Comment in PMID 7908847 Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The proportion of peripheral blood mononuclear cells expressing the T helper cell phenotype and levels of antiendotoxin core antibody, interleukin (*IL*) *6* and C-reactive protein (CRP) were determined within 48 h of admission in a group of 29 patients with acute *pancreatitis* (16 mild, 13 severe attacks). There was a significant decrease in the proportion of T helper cells (12.2 versus 34.9 per cent, P < 0.01) and significant increases in levels of *IL*-*6* (69.5 versus < 10 pg/ml, P < 0.01) and CRP (119 versus 30.5 mg/l, P < 0.01) in severe compared with mild attacks. During the convalescent stage at 3 months after admission, severe attacks were characterized by a significant increase in the proportion of T helper cells compared with the acute period (22.4 versus 10.6 per cent, P < 0.01). A persistently low proportion of T helper cells was associated with residual pancreatic necrosis. The presence of circulating endotoxin was demonstrated in two mild and two severe attacks using the Limulus amoebocyte lysate assay, and abnormal levels of antiendotoxin core antibodies were found in 70 and 92 per cent of mild and severe attacks respectively. There was a strong inverse correlation between levels of CRP and the proportion of T helper cells in severe disease (r = -0.76, P = 0.004). Translocation of endotoxin from the gastrointestinal

tract may partly explain the abnormal levels of T helper cells, *IL*- *6* and CRP.

11/3,AB/51 (Item 51 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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07918139 93378971 PMID: 8369291
Identification of a second rat *pancreatitis*-associated protein. Messenger RNA cloning, gene structure, and expression during acute *pancreatitis*.
Frigerio J M; Dusetti N J; Keim V; Dagorn J C; Iovanna J L U.315 INSERM, Marseille, France.
Biochemistry (UNITED STATES) Sep 7 1993, 32 (35) p9236-41, ISSN 0006-2960 Journal Code: 0370623
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The *pancreatitis* -associated protein (PAP) is a lectin-related secretory protein present in small amounts in the rat pancreas and overexpressed during the acute phase of *pancreatitis*. On the other hand, PAP is constitutively expressed in the intestinal tract but not in other tissues. We cloned from a pancreatic cDNA library two overlapping cDNAs encoding a protein structurally related to PAP. This second PAP, which was called PAP II, was the same size as the original PAP (PAP I) and showed 74.3% amino acid homology. Studies on gene expression demonstrated that PAP II mRNA concentration increased within 6 h following induction of *pancreatitis* , reached maximal levels (> 200 times control values) at 24-48 h, and decreased thereafter, similar to PAP I. However, PAP II mRNA could not be detected in the intestinal tract or in other tissues. We also isolated a PAP II genomic DNA fragment which was characterized over 2.7 kb of gene sequence and 1.9 kb of 5' flanking sequence. The 5' end of the coding sequence was determined by primer extension of the PAP II mRNA. The PAP II coding sequence spanned six exons separated by five introns. Several potential regulatory elements were identified in the promoter region, including two glucocorticoid-response elements and one *IL*-*6* -response element. Antibodies raised to a synthetic peptide of PAP II detected a single band in Western blot analysis of the pancreatic secretory proteins from rats with *pancreatitis*, with a M(r) compatible with the theoretical M(r) of PAP II.(ABSTRACT TRUNCATED AT 250 WORDS)

11/3,AB/52 (Item 52 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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07845179 93300847 PMID: 8314803
Structural organization of the gene encoding the rat *pancreatitis* -associated protein. Analysis of its evolutionary history reveals an ancient divergence from the other carbohydrate-recognition domain-containing genes.
Dusetti N J; Frigerio J M; Keim V; Dagorn J C; Iovanna J L U.315 Institut National de la Sante et de la Recherche Medicale, Marseille, France.
Journal of biological chemistry (UNITED STATES) Jul 5 1993, 268 (19) p14470-5, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Rat *pancreatitis*-associated protein (PAP) mRNA is barely detectable in normal pancreas and overexpressed during acute *pancreatitis* (Iovanna, J., Orelle, B., Keim, V., and Dagorn J.-C. (1991) J. Biol. Chem. 266, 24664-24669). RNA amplification by reverse-transcriptase-coupled polymerase chain reaction showed that PAP mRNA was constitutively expressed in duodenum, jejunum, and ileum, at similar levels as in pancreas during the acute phase of *pancreatitis*. A weak expression was also detected in several other tissues. The rat PAP gene was isolated from a genomic library and characterized over 3.2 kilobases of gene sequence and 1.2 kilobases of 5'-flanking sequence. The 5' end of the coding sequence was determined by primer extension of the PAP transcript. Several potential regulatory elements were identified in the promoter region, including a pancreas-specific consensus sequence, two Pan1 (pancreas-specific) transcription activators, two *IL*-*6* response elements, and one glucocorticoid response element. The PAP coding sequence spanned over six exons. The first three exons encoded the 5'-untranslated region of the mRNA, the signal peptide, and 39 amino acids of the NH2-terminal end of the mature protein, respectively. The other three exons encoded a domain of the protein with significant homology to the carbohydrate-recognition domain of animal lectins. Sequence comparison of the PAP gene with 13 carbohydrate-recognition domain-containing genes revealed that they derived from the same ancestor gene. Position of introns within the carbohydrate-recognition domain were different, however, suggesting that PAP belongs to a new group of lectins. These results support the hypothesis that genes encoding PAP and other lectins evolved from a common ancestor gene by intron gain.

11/3,AB/53 (Item 53 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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07512793 92376500 PMID: 1509242
Endotoxin, TNF-alpha, interleukin-6 and parameters of the cellular immune system in patients with intraabdominal sepsis.
Hamilton G; Hofbauer S; Hamilton B
First Department of Surgery, University School of Medicine, Vienna, Austria.
Scandinavian journal of infectious diseases (SWEDEN) 1992, 24 (3) p361-8, ISSN 0036-5548 Journal Code: 0215333
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The correlation of endotoxin (ET), tumor necrosis factor alpha (TNF-alpha), interleukin-6 (*IL*-*6*), and cellular immune parameters with multiple organ failure and lethal outcome in intraabdominal infections was studied in a group of 18 patients with peritonitis, abscess or *pancreatitis* . Of these patients, 7 developed respiratory failure and 5 died due to multiple septic organ failure. The peak levels of ET (2.7 +/- 1.3 ng/ml) in the course of the disease were followed by moderate increases of TNF-alpha (mean 147 +/- 41 pg/ml) and *IL*-*6* (170 +/- 61 pg/ml) within 2 days. Analysis of the parameters for the last 12 days prior to death or discharge showed, that the patient group with lethal outcome was characterized by significant lower mean plasma levels of TNF-alpha (less than 75 pg/ml versus greater than 160 pg/ml) and *IL*- *6* (less than 130 pg/ml versus greater than 270 pg/ml), as well as high rates of unstimulated thymidine uptake into peripheral mononuclear blood cells (greater than 44000 cpm/8 x 10(6) PMBC/18 h versus less than 24000 cmp), T-lymphocyte depression (CD3; approximately greater than 40% reduction) with lower T-helper/inducer subset cell numbers (mean CD:CD8 ratio 1.0 +/- 0.55 versus 1.8 +/- 0.2) and lower lectin (PHA) stimulation values (1.9 +/- 1.4 versus 4.1 +/- 1.0). These data demonstrate an anergic immune status with low mediator levels and depressed T-lymphocyte function in patients with poor prognosis.(ABSTRACT TRUNCATED AT 250 WORDS)

11/3,AB/54 (Item 54 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.

06681232 90307119 PMID: 2114355
Serum interleukin 6, C-reactive protein and pancreatic secretory trypsin inhibitor (PSTI) as acute phase reactants after major thoraco-abdominal surgery.
Murata A; Ogawa M; Yasuda T; Nishijima J; Oka Y; Ohmachi Y; Hiraoka N; Niinobu T; Uda K; Mori T
Second Department of Surgery, Osaka University Medical School, Japan. Immunological investigations (UNITED STATES) Jun 1990, 19 (3) p271-8 , ISSN 0882-0139 Journal Code: 8504629
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We measured serum immunoreactive interleukin 6 (*IL*-*6*) levels in patients after major thoraco-abdominal surgery and compared them with changes in serum C-reactive protein (CRP) and pancreatic secretory trypsin inhibitor (PSTI) levels. Serum *IL*-*6* levels were elevated earlier than serum CRP or PSTI. There were significant relationships between the peak levels of serum *IL*-*6* and the operation time (p less than 0.05) or the volume of blood loss during surgery (p less than 0.05), showing that serum *IL*-*6* can be a useful laboratory test for the evaluation of tissue injuries due to surgical intervention. Though there was a significant relationship between the peak levels of *IL*- *6* and CRP (p less than 0.01), there was no relationship between the peak levels of *IL*-*6* and PSTI.

11/3,AB/55 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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11715857 BIOSIS NO.: 199800497588
Complex treatment of infected pancreatic necrosis.
AUTHOR: Farkas Gyula(a); Marton Janos; Mandi Yvette; Nagy Erzsebet; Szederkenyi Edit
AUTHOR ADDRESS: (a)Pecsi u. 4, 6720 Szeged**Hungary
JOURNAL: Orvosi Hetilap 139 (38):p2235-2240 Sept. 20, 1998 ISSN: 0030-6002
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: Hungarian; Non-English
SUMMARY LANGUAGE: Hungarian; English

ABSTRACT: Pancreatic necrosis associated with septic conditions is the leading cause of mortality in acute *pancreatitis*. Since 1986, 155 patients with infected pancreatic necrosis have been treated. The mean APACHE II score was 18.5 (range 11-32). In all cases, the infected pancreatic necrosis was combined with retroperitoneal abscesses. The surgical treatment was performed on average 18.5 days (range 8-25 days) after the onset of acute *pancreatitis*. The operative management consisted of wide-ranging necrosectomy in the total affected area, combined with widespread lavage and suction drainage. In 69 of the 155 cases (45%), some other surgical intervention (distal pancreatic resection, splenectomy, cholecystectomy, sphincteroplasty or colon resection) was also performed.

Following surgery supportive therapy was applied in all patients, which also consisted orf immunonutrition (glutamine and arginine supplementation) and modification of cytokine production by petoxyfillin and dexamethasone from 1992. TNF and *IL* -*6* serum levels were measured by ELISA and in vitro stimulation of leukocytes were induced by E. coli LPS. Following surgery, continuous lavage and suction drainage were applied for an average of 41.5 days (range 21-90 days), with an average of 9.5 (range 5-20) litres of saline per day. The bacteriologic findings revealed mainly enteral bacteria, but Candida infection was also frequently detected. The incidence of fungal infection was 20%. Thirty-two patients (21%) had to undergo reoperation. The cytokine production capacity (TNF and *IL*-*6*) was shown to correlate with the prognosis. As a consequence of pentoxifyllin and dexamethasone therapy, the TNF production generally dropped to the normal level. The overall hospital mortality was 6.4% (10 patients died). in our experience, infected pancreatic necrosis responds well to aggressive surgical treatment, continuous, long-standing lavage and suction drainage, together with supportive therapy consisting of immunonutrition and modification of cytokine production, combined with adequate antibiotic and antifungal medication.

1998

11/3,AB/56 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
(c) 2003 BIOSIS. All rts. reserv.

11504749 BIOSIS NO.: 199800286081
IL-*6* and CRP levels as predictive factors for post-ERCP *pancreatitis*.
AUTHOR: Zagoni T; Pronai L; Papik K; Kope A; Nemeth A; Tulassay Z AUTHOR ADDRESS: 2nd Dep. Intern. Med., Semmelweis Med. Univ., Hungarian Acad. Sci., Clin. Gastroenterol. Res. Unit, **Hungary
JOURNAL: Gastroenterology 114 (4 PART 2):pA512 April 15, 1998 CONFERENCE/MEETING: Digestive Disease Week and the 99th Annual Meeting of the American Gastroenterological Association New Orleans, Louisiana, USA May 16-22, 1998
SPONSOR: American Gastroenterological Association
ISSN: 0016-5085
RECORD TYPE: Citation
LANGUAGE: English
1998

11/3,AB/57 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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11504631 BIOSIS NO.: 199800285963
Pyridium cross links, *IL*-*6* and IL-8 levels in acute *pancreatitis*.
AUTHOR: Miah A; Nandi S; Bailey B; Bank S
AUTHOR ADDRESS: Div. Gastroenterol., Long Island Jewish Med. Cent., New Hyde Park, NY**USA
JOURNAL: Gastroenterology 114 (4 PART 2):pA483 April 15, 1998 CONFERENCE/MEETING: Digestive Disease Week and the 99th Annual Meeting of the American Gastroenterological Association New Orleans, Louisiana, USA May 16-22, 1998
SPONSOR: American Gastroenterological Association
ISSN: 0016-5085
RECORD TYPE: Citation
LANGUAGE: English
1998

11/3,AB/58 (Item 4 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
(c) 2003 BIOSIS. All rts. reserv.

11504465 BIOSIS NO.: 199800285797
Intrapancreatic expression of cytokines *IL*-*6* and KC is increased in cerulein *pancreatitis*: Regulation by NF-kappaB. AUTHOR: Blinman T A(a); Gukovsky I; Gukovskaya A; Zaninovic V; Pandol S J; Livingston E
AUTHOR ADDRESS: (a)Univ. California Los Angeles, Dep. Surg., Los Angeles, CA**USA
JOURNAL: Gastroenterology 114 (4 PART 2):pA443 April 15, 1998 CONFERENCE/MEETING: Digestive Disease Week and the 99th Annual Meeting of the American Gastroenterological Association New Orleans, Louisiana, USA May 16-22, 1998
SPONSOR: American Gastroenterological Association
ISSN: 0016-5085
RECORD TYPE: Citation
LANGUAGE: English
1998

11/3,AB/59 (Item 5 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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11504464 BIOSIS NO.: 199800285796
Pancreatic acinar cells express *IL*-*6*, KC, MCP-1, and MIP-2: Evidence from RT-PCR analysis of microscopically isolated acinar cells. AUTHOR: Blinman T(a); Gukovskaya A; Gukovsky I; Zaninovic V; Livingston E; Pandol S J
AUTHOR ADDRESS: (a)Univ. California Los Angeles Dep. Surg., Los Angeles, CA **USA
JOURNAL: Gastroenterology 114 (4 PART 2):pA443 April 15, 1998 CONFERENCE/MEETING: Digestive Disease Week and the 99th Annual Meeting of the American Gastroenterological Association New Orleans, Louisiana,

USA May 16-22, 1998
SPONSOR: American Gastroenterological Association
ISSN: 0016-5085
RECORD TYPE: Citation
LANGUAGE: English
1998

11/3,AB/60 (Item 6 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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11472833 BIOSIS NO.: 199800254165
Evaluation of phospholipase A|2 measurement for early prediction of the severity of acute *pancreatitis*.
AUTHOR: Inagaki Takanori; Hoshino Makoto; Ohara Hirotaka; Yamada Tamaki AUTHOR ADDRESS: First Dep. Intern. Med., Nagoya City Univ. Med. Sch., Nagoya**Japan
JOURNAL: Pancreas 16 (4):p558-559 May, 1998
ISSN: 0885-3177
DOCUMENT TYPE: Letter
RECORD TYPE: Citation
LANGUAGE: English
1998

11/3,AB/61 (Item 7 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
(c) 2003 BIOSIS. All rts. reserv.

11451645 BIOSIS NO.: 199800232977
Beneficial effects of pentoxyfylline treatment of experimental acute *pancreatitis* in rats.
AUTHOR: Marton J(a); Farkas G; Takacs T; Nagy Z; Szasz Z; Varga J; Jarmay K ; Balogh A; Lonovics J
AUTHOR ADDRESS: (a)Dep. Surg., Albert Szent-Gyorgyi Med. Univ., POB 469, H-6701 Szeged**Hungary
JOURNAL: Research in Experimental Medicine 197 (5):p293-299 March, 1998 ISSN: 0300-9130
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The purposes of this study were to determine the tumor necrosis factor (TNF) and interleukin-6 (*IL*-*6*) levels after the induction of acute necrotizing *pancreatitis*, and to establish the effects of pentoxifylline on cytokine production. Methods: acute *pancreatitis* was induced by the retrograde injection of 200 mul taurocholic acid into the pancreatic duct in male Wistar rats. The serum amylase activity, the wet pancreatic weight/body weight ratio, and the TNF and *IL*-*6* levels were measured. Seven mg/kg pentoxifylline were administered intraperitoneally at the time of operation 6, 12 or 24 h later. Rats were killed 6, 24, 48 or 72 h after the operation. Results: the TNF bioassay revealed high levels of TNF (30.2 +- 5.4 U/ml, 35.0 +- 5.0 U/ml and 36.6 +- 6.0 U/ml) in the control group at 6, 24 and 48 h and (54.1 +- 20 U/ml and 10.9 +- 4.2 U/ml) in the pentoxifylline-treated group at 6 and 24 h, respectively, whereas the level had decreased to zero in the pentoxifylline-treated group at 48 h. The *IL*-*6* bioassay likewise demonstrated high levels of *IL*-*6* in the control group at 48 h and in the pentoxifylline-treated group at 6 and 24 h, and markedly decreased levels in the pentoxifylline-treated group at 48 h (7083 +- 2844 pg/ml, 6463 +- 1307 pg/ml, 10329 +- 5571 pg/ml vs 137.5 +- 85.5 pg/ml, respectively, P < 0.05). The high mortality observed in the *pancreatitis* group (43%) was decreased by pentoxifylline administration to 11%. Conclusion: these results demonstrate that pentoxifylline very effectively inhibits cytokine production in acute *pancreatitis*.

1998

11/3,AB/62 (Item 8 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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11364327 BIOSIS NO.: 199800145659
Early treatment with the platelet activating factor antagonist lexipafant reduces mortality and markers of leucocyte and endothelial activation. AUTHOR: Wilson P(a); Scott E(a); Neoptolemos J P
AUTHOR ADDRESS: (a)Univ. Birmingham, Queen Elizabeth Med. Cent., Birmingham **UK
JOURNAL: Pancreas 15 (4):p461 Nov., 1997
CONFERENCE/MEETING: Meeting of the American Pancreatic Association Chicago, Illinois, USA November 6-8, 1997
SPONSOR: American Pancreatic Association
ISSN: 0885-3177
RECORD TYPE: Citation
LANGUAGE: English
1997

11/3,AB/63 (Item 9 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
(c) 2003 BIOSIS. All rts. reserv.

11364314 BIOSIS NO.: 199800145646
The effects of glucocorticoids and a glucocorticoid antagonist (RU 38486) on experimental acute *pancreatitis* in rat.
AUTHOR: Takacs T(a); Lazar G Jr; Varga J(a); Lazar G; Duda E; Balogh A; Lonovics J(a)
AUTHOR ADDRESS: (a)First Dep. Med., Albert Szent-Gyorgyi Med. Univ., Szeged **Hungary

JOURNAL: Pancreas 15 (4):p458 Nov., 1997
CONFERENCE/MEETING: Meeting of the American Pancreatic Association Chicago, Illinois, USA November 6-8, 1997
SPONSOR: American Pancreatic Association
ISSN: 0885-3177
RECORD TYPE: Citation
LANGUAGE: English
1997

11/3,AB/64 (Item 10 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
(c) 2003 BIOSIS. All rts. reserv.

11364264 BIOSIS NO.: 199800145596
Increased cytokines in pulmonary and renal dysfunction in acute *pancreatitis*.
AUTHOR: Mayer J; Rau B; Schoenberg M H; Beger H G
AUTHOR ADDRESS: Dep. Gen. Surg., Univ. Hosp., D-89075 Ulm**Germany JOURNAL: Pancreas 15 (4):p445 Nov., 1997
CONFERENCE/MEETING: Meeting of the American Pancreatic Association Chicago, Illinois, USA November 6-8, 1997
SPONSOR: American Pancreatic Association
ISSN: 0885-3177
RECORD TYPE: Citation
LANGUAGE: English
1997

11/3,AB/65 (Item 11 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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11183496 BIOSIS NO.: 199799804641
Peptide YY suppresses serum *IL*-*6* and TNF-alpha levels in murine necrotizing *pancreatitis*.
AUTHOR: Reed Kimberlee(a); McFadden David
AUTHOR ADDRESS: (a)Dep. Surg., UCLA Med. Cent., Los Angeles, CA**USA JOURNAL: Surgical Forum 48 (0):p179-180 1997
ISSN: 0071-8041
RECORD TYPE: Citation
LANGUAGE: English
1997

11/3,AB/66 (Item 12 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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11162837 BIOSIS NO.: 199799783982
Expression of oxidative stress-responsive genes and cytokine genes during caerulein-induced acute *pancreatitis*.
AUTHOR: Fu Kai; Sarras Michael P Jr; De Lisle Robert C; Andrews Glen K(a) AUTHOR ADDRESS: (a)Dep. Biochemistry Molecular Biol., BRF 2034, Univ. Kansas Med. Cent., 39th Rainbow Blvd., Kansas**USA
JOURNAL: American Journal of Physiology 273 (3 PART 1):pG696-G705 1997 ISSN: 0002-9513
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Oxidative stress and the inflammatory response may play roles in the pathogenesis of acute *pancreatitis*. Herein, we characterized pancreatic expression of oxidative stress-responsive genes (c-fos, heme oxygenase-1 (HO-1), and metallothionein-I (MT-I)) and cytokine genes (interleukin-1-beta (IL-1-beta), *IL*-*6*, and tumor necrosis factor-alpha (TNF-alpha)) during caerulein-induced acute *pancreatitis* in the mouse. c-fos, HO-1, and MT-1 mRNAs were coordinately and rapidly (3-7 h) upregulated, and HO-1 and MT-I protein levels were increased slightly in the pancreas during acute *pancreatitis*. In addition, IL-1-beta, *IL*-*6*, and TNF-alpha mRNAs were rapidly (7 h) upregulated in the pancreas, and intrapancreatic IL-1-beta and *IL*-*6* protein levels rapidly increased (3-fold and 6.4-fold, respectively) during acute *pancreatitis*. These studies suggest that oxidative stress and inflammation each occur in the pancreas during the early stages of acute *pancreatitis*, However, under a limited set of experimental conditions, we found that an insult that causes pancreatic oxidative stress (diethylmaleate) or one that induces an inflammatory response (bacterial lipopolysaccharide), or a combination of these agents, did not cause the changes characteristic of acute *pancreatitis*. Therefore, simply inducing oxidative stress and/or inflammation may be insufficient to initiate acute *pancreatitis*.

1997

11/3,AB/67 (Item 13 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
(c) 2003 BIOSIS. All rts. reserv.

11155376 BIOSIS NO.: 199799776521
An investigation into the inflammatory response following simultaneous total pancreatectomy and intraportal islet autotransplantation. AUTHOR: Davies J E; White S A; Clayton H A; Swift S M; Dennison A R AUTHOR ADDRESS: Dep. General Surgery, Leicester General Hospital, Gwendolen Road, Leicester LE5 4PW**UK
JOURNAL: Acta Diabetologica 34 (2):p147 1997
CONFERENCE/MEETING: 6th Congress of the International Pancreas and Islet Transplant Association (IPITA) 1997 Milan, Italy September 24-27, 1997

ISSN: 0940-5429
RECORD TYPE: Citation
LANGUAGE: English
1997

11/3,AB/68 (Item 14 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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11104089 BIOSIS NO.: 199799725234
Severe systemic response syndrome in acute *pancreatitis* is mediated by disbalanced cytokines.
AUTHOR: Mayer J; Rau B; Schoenberg M H; Beger H G
AUTHOR ADDRESS: Dep. General Surg., Univ. Hosp., Ulm**Germany JOURNAL: Digestion 58 (SUPPL. 2):p59 1997
CONFERENCE/MEETING: 29th European Pancreatic Club Meeting London, England, UK July 9-12, 1997
ISSN: 0012-2823
RECORD TYPE: Citation
LANGUAGE: English
1997

11/3,AB/69 (Item 15 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
(c) 2003 BIOSIS. All rts. reserv.

11103988 BIOSIS NO.: 199799725133
Expression of calcium binding proteins during the course of acute *pancreatitis*.
AUTHOR: Chikh-Torab F; Berger D; Beger H G
AUTHOR ADDRESS: Univ. Hosp., Ulm**Germany
JOURNAL: Digestion 58 (SUPPL. 2):p34 1997
CONFERENCE/MEETING: 29th European Pancreatic Club Meeting London, England, UK July 9-12, 1997
ISSN: 0012-2823
RECORD TYPE: Citation
LANGUAGE: English
1997

11/3,AB/70 (Item 16 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
(c) 2003 BIOSIS. All rts. reserv.

11103902 BIOSIS NO.: 199799725047
Expression of immunoregulatory cytokines in pancreatic duct cell lines. AUTHOR: Emmrich J(a); Wilden V(a); Sparmann G(a); Loehr M(a); Vu L H; Moldoveanu Z; Mestecky J; Liebe S(a)
AUTHOR ADDRESS: (a)Dep. Med., Univ. Rostock, Rostock**Germany JOURNAL: Digestion 58 (SUPPL. 2):p12 1997
CONFERENCE/MEETING: 29th European Pancreatic Club Meeting London, England, UK July 9-12, 1997
ISSN: 0012-2823
RECORD TYPE: Citation
LANGUAGE: English
1997

11/3,AB/71 (Item 17 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
(c) 2003 BIOSIS. All rts. reserv.

10956830 BIOSIS NO.: 199799577975
Cytokine mediated T-cell activation in acute *pancreatitis*. AUTHOR: Mayer J; Rau B; Kolodziej S; Beger H G
AUTHOR ADDRESS: General Surgery, Univ. Ulm, Ulm**Germany
JOURNAL: Gastroenterology 112 (4 SUPPL.):pA463 1997
CONFERENCE/MEETING: Digestive Disease Week and the 97th Annual Meeting of the American Gastroenterological Association Washington, D.C., USA May 11-14, 1997
ISSN: 0016-5085
RECORD TYPE: Citation
LANGUAGE: English
1997

11/3,AB/72 (Item 18 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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10956755 BIOSIS NO.: 199799577900
Kupffer cell activation increases serum cytokines in a model of acute *pancreatitis*.
AUTHOR: Gloor B(a); Todd K E; Lane J S; Lewis M P N; Reber H A AUTHOR ADDRESS: (a)Dep. Surgery, UCLA Sch. Med., Los Angeles, CA**USA JOURNAL: Gastroenterology 112 (4 SUPPL.):pA444 1997
CONFERENCE/MEETING: Digestive Disease Week and the 97th Annual Meeting of the American Gastroenterological Association Washington, D.C., USA May 11-14, 1997
ISSN: 0016-5085
RECORD TYPE: Citation
LANGUAGE: English
1997

11/3,AB/73 (Item 19 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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10712070 BIOSIS NO.: 199799333215
Detection of interleukin-6 in serum of patients with diabetic ketoacidosis complicated with acute

pancreatitis in two cases.
AUTHOR: Seino Hiroaki; Hirata Akihiko; Yamaguchi Hiroshi; Yamazaki Toshio; Kikuchi Hiroaki; Abe Ryuzo
AUTHOR ADDRESS: Dep. Intern. Med., Diabetic Cent., Ohta Nishinouchi Hosp., 2-5-20 Nishinouchi, Koriyama 963**Japan
JOURNAL: Journal of the Japan Diabetes Society 39 (9):p721-727 1996 ISSN: 0021-437X
RECORD TYPE: Abstract
LANGUAGE: Japanese; Non-English
SUMMARY LANGUAGE: Japanese; English

ABSTRACT: We have experienced some patients with diabetic ketoacidosis complicated with acute *pancreatitis*, who had elevated serum levels of amylase, lipase and elastase-1. In case 1, a 46 year-old man was admitted to our hospital because of diabetic ketoacidosis. Laboratory findings revealed a blood glucose level of 763 mg/dl and a pH level of 6.93. In case 2, a 43 year-old man was admitted because of diabetic ketocidosis, and laboratory findings revealed a blood glucose level of 1573 and pH levels of 7.19. During the treatment of diabetic ketoacidosis, the maximum serum levels of amylase and lipase were 762 u/l and 520 u/l respectively in case 1, and the maximum serum levels of amylase, elastase-1 and pancreatic phospholipase-A2 were 2107 u/l, 8310 ng/dl and 34300 ng/dl respectively in case 2. We measured the serum cytokine levels of *IL*-*6*, IL-1-beta and GM-CSF in these two cases by ELISA. Only *IL*-*6* was detected in the serum of the two patients with diabetic ketoacidosis complicated with acute *pancreatitis*. The maximum serum level of *IL*-*6* was 380 pg/ml in case 1 and 80 pg/ml in case 2. In the other two cases of diabetic ketoacidosis which was not complicated with acute *pancreatitis*. the serum levels of *IL*-*6* were at the lower levels of 14 pg/ml and 11 pg/ml. Serum *IL*-*6* in patients with diabetic ketoacidosis may induce acute *pancreatitis*. According to our knowledge, this is the first report of detection of *IL*- *6* in the serum of patients with diabetic ketoacidosis complicated with acute *pancreatitis*.

1996

11/3,AB/74 (Item 20 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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10399750 BIOSIS NO.: 199699020895
In vitro secretion of bioactive tumor necrosis-alpha (TNF-alpha) and interleukin-6 (*IL*-*6*) by leukocytes in patients with alcoholic liver diseases and chronic calcific *pancreatitis*.
AUTHOR: Nagy I(a); Mandi Y; Takacs T(a); Lonovics J(a)
AUTHOR ADDRESS: (a)1st Dep. Med., A Szent-Gyorgyi Med. Univ., Szeged** Hungary
JOURNAL: Gastroenterology 110 (4 SUPPL.):pA419 1996
CONFERENCE/MEETING: 96th Annual Meeting of the American Gastroenterological Association and the Digestive Disease Week San Francisco, California, USA May 19-22, 1996
ISSN: 0016-5085
RECORD TYPE: Citation
LANGUAGE: English
1996

11/3,AB/75 (Item 21 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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09561826 BIOSIS NO.: 199598016744
Biliary interleukin-6 (*IL*-*6*) and tumor necrosis factor (TNF-alpha) in patients undergoing ERCP.
AUTHOR: Rosen H R; Winkle P J; Kendall B J; Diehl D L; So L; Jamidar P; Batah J
AUTHOR ADDRESS: Dep. Dig. Dis., UCLA Med. Cent., Los Angeles, CA**USA JOURNAL: Hepatology 20 (4 PART 2):p118A 1994
CONFERENCE/MEETING: 45th Annual Meeting of the American Association for the Study of Liver Diseases Chicago, Illinois, USA November 11-15, 1994 ISSN: 0270-9139
RECORD TYPE: Citation
LANGUAGE: English
1994

11/3,AB/76 (Item 22 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
(c) 2003 BIOSIS. All rts. reserv.

09469606 BIOSIS NO.: 199497477976
In vitro production of tumor necrosis-alpha (TNF-alpha) and interleukin-6 (*IL*-*6*) by leukocytes in patients with alcoholic liver diseases and chronic calcific *pancreatitis*.
AUTHOR: Nagy I(a); Mandi Y; Takacs T(a); Lonovics J(a)
AUTHOR ADDRESS: (a)1st Dep. Med., A. Szent-Gyorgyi Med. Univ., Szeged** Hungary
JOURNAL: Digestion 55 (5):p319 1994
CONFERENCE/MEETING: XXVIth Meeting of the European Pancreatic Club Bologna, Italy September 7-10, 1994
ISSN: 0012-2823
RECORD TYPE: Citation
LANGUAGE: English
1994

11/3,AB/77 (Item 23 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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09469592 BIOSIS NO.: 199497477962
Usefulness of interleukin-6 (*IL*-*6*) in the early assessment of the severity of acute biliary *pancreatitis*.
AUTHOR: Manes G(a); Spada O; Uomo G; Iannaccone L; Laccetti M(a); Pacelli L (a); Rabitti P G(a); Visconti M(a)
AUTHOR ADDRESS: (a)Pancreatic Disease Center, Internal Med., Cardarelli Hosp., Napoli**Italy
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CORRELATION OF ELEVATED SERUM LEVELS OF INTERLEUKIN-6 *IL*-*6* AND C-REACTIVE PROTEIN IN PATIENTS WITH ACUTE *PANCREATITIS*
AUTHOR: LESER H-G; GROSS V; SCHEIBENBOGEN C; HEINISCH A; SALM R; LAUSEN M; RUECKAUER K; ANDREESEN R; FARTHMANN E H; ET AL
AUTHOR ADDRESS: DEP. MED., UNIV. FREIBURG, D-7800 FREIBURG, W. GER. JOURNAL: 91ST ANNUAL MEETING OF THE AMERICAN GASTROENTEROLOGICAL ASSOCIATION AND DIGESTIVE DISEASE WEEK, SAN ANTONIO, TEXAS, USA, MAY 12-18, 1990. GASTROENTEROLOGY 98 (5 PART 2). 1990. A224. 1990
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S2 545 PM()1
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Characterization of anti-mouse interleukin-6 receptor *antibody*. Okazaki Makoto; Yamada Yoshiki; Nishimoto Norihiro; Yoshizaki Kazuyuki; Mihara Masahiko
Fuji-Gotemba Research Laboratories, Chugai Pharmaceutical Co. Ltd, 135 Komakado 1-chome, Gotemba-shi, Shizuoka 412-8513, Japan.
Immunology letters (Netherlands) Dec 3 2002, 84 (3) p231-40, ISSN 0165-2478 Journal Code: 7910006
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10) Blockage of interleukin-6 receptor ameliorates joint disease in murine collagen-induced arthritis.
Takagi N; Mihara M; Moriya Y; Nishimoto N; Yoshizaki K; Kishimoto T; Takeda Y; Ohsugi Y
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12) Soluble interleukin-6 receptor triggers osteoclast formation by interleukin 6.
Tamura T; Udagawa N; Takahashi N; Miyaura C; Tanaka S; Yamada Y; Koishihara Y; Ohsugi Y; Kumaki K; Taga T; et al
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Research in experimental medicine. Zeitschrift fur die gesamte experimentelle Medizin einschliesslich experimenteller Chirurgie (GERMANY) 1998 197 (5) p293-9, ISSN 0300-9130 Journal Code: 0324736 Document type: Journal Article

14) Experimental acute *pancreatitis* results in increased blood-brain barrier permeability in the rat: a potential role for tumor necrosis factor and interleukin 6.
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Neuroscience letters (IRELAND) Feb 20 1998, 242 (3) p147-50, ISSN 0304-3940 Journal Code: 7600130
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16) Peptide YY suppresses serum *IL*-*6* and TNF-alpha levels in murine necrotizing *pancreatitis*.
AUTHOR: Reed Kimberlee(a); McFadden David
AUTHOR ADDRESS: (a)Dep. Surg., UCLA Med. Cent., Los Angeles, CA**USA JOURNAL: Surgical Forum 48 (0):p179-180 1997
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Beneficial effects of pentoxifylline treatment of experimental acute pancreatitis in rats

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Abstract. The purposes of this study were to determine the tumor necrosis factor (TNF) and interleukin-6 (IL-6) levels after the induction of acute necrotizing pancreatitis, and to establish the effects of pentoxifylline on cytokine production. Methods: acute pancreatitis was induced by the retrograde injection of 200 µl taurocholic acid into the pancreatic duct in male Wistar rats. The serum amylase activity, the wet pancreatic weight/body weight ratio, and the TNF and IL-6 levels were measured. Seven mg/kg pentoxifylline were administered intraperitoneally at the time of operation 6, 12 or 24 h later. Rats were killed 6, 24, 48 or 72 h after the operation. Results: the TNF bioassay revealed high levels of TNF (30.2±5.4 U/ml, 35.0±5.0 U/ml and 36.6±6.0 U/ml) in the control group at 6, 24 and 48 h and (54.1±20 U/ml and 10.9±4.2 U/ml) in the pentoxifylline-treated group at 6 and 24 h, respectively, whereas the level had decreased to zero in the pentoxifylline-treated group at 48 h. The IL-6 bioassay likewise demonstrated high levels of IL-6 in the control group at 48 h and in the pentoxifylline-treated group at 6 and 24 h, and markedly decreased levels in the pentoxifylline-treated group at 48 h (7083±2844 pg/ml, 6463±1307 pg/ml, 10329±5571 pg/ml vs 137.5± 85.5 pg/ml, respectively, $P < 0.05$). The high mortality observed in the pancreatitis group (43%) was decreased by pentoxifylline administration to 11%. Conclusion: these results demonstrate that pentoxifylline very effectively inhibits cytokine production in acute pancreatitis.

Key words: Experimental acute pancreatitis – Tumor necrosis factor – Interleukin-6 – Pentoxifylline

Introduction

In approximately 10% of all cases acute pancreatitis can turn into severe or lethal acute necrotizing pancreatitis. Despite numerous clinical and experi-

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mental investigations, it remains very difficult to predict the outcome of individual cases [6, 10]. It has been proposed that major surgical operation, trauma, burns, and sepsis cause excessive leukocyte activation and the overproduction of proinflammatory cytokines such as TNF (tumor necrosis factor) and IL-6 (interleukin-6) [9, 17, 18]. This cascade of events can lead to the development of a systemic inflammatory response and multi-organ failure, independently of the origin of the process [19]. Moreover, genetic determination of the host response may also influence the magnitude of the reaction to the initial event [15]. Major tissue necrosis and systemic vascular and metabolic disorders are evident in cases of severe necrotizing pancreatitis, especially when infection exacerbates the clinical picture. These common features of systemic inflammatory response and severe acute necrotizing pancreatitis reasonably suggest that cytokines, and especially TNF, may play a pivotal role in the pathogenesis of the above-mentioned processes [8, 12]. Decrease of the cytokine response by blocking their release or neutralizing them is a very promising concept [13, 14]. Pentoxifylline, a methylxanthine derivative, has been used worldwide for the treatment of patients with intermittent claudication by virtue of its rheologic effect. Recent studies have revealed that pentoxifylline not only influences the blood viscosity and flow, but also has some immunologic properties, e. g., it can decrease monocyte TNF production [16]. Recent evidence suggests that transcription of TNF mRNA is blocked by pentoxifylline [4]. Accordingly, the possible beneficial effect of anti-cytokine treatment appears reasonable [7]. The course and severity of experimental pancreatitis induced by the intraductal injection of taurocholic acid, described by Aho et al. [1] are very similar to those in human acute necrotizing pancreatitis. We therefore selected this model for our experimental investigations. Our study was designed to determine the changes in the serum levels of TNF and IL-6 with time and to examine the influence of pentoxifylline administration on the cytokine production in this experimental model of acute pancreatitis.

Materials and methods

Male Wistar rats weighing 200–260 g were used in all experiments. The animals were kept at a constant room temperature of 27°C, with free access to water and a standard laboratory chow (LATI, Gödöllő, Hungary). The experiment followed the Principles of Laboratory Animal Care of the NIH. Acute pancreatitis was induced in accordance with Aho et al. [1]. Ether anesthesia was induced, the abdomen was shaved, prepared and draped in a sterile fashion, and a midline incision was made. The pancreatic duct was cannulated transduodenally and the common bile duct was temporarily closed with a metal clamp. A knot was tightened around the pancreatic duct and the cannula. Next 200 µl 6% taurocholic acid (Reanal, Hungary) was injected via the cannula for 1 min. After infusion, the clamp, knot and cannula were removed and the duodenal wound was closed with a single figure-of-eight 6–0 Prolene suture. Sham-operated animals underwent laparotomy and exploration of the duodenum and pancreas. The pentoxifylline-treated group received 7 mg/kg pentoxifylline intraperitoneally at the time of operation and 6, 12, or 24 h later. Treated and untreated animals and sham-operated animals were killed 6, 24, 48 or 72 h after the operation. Wet pancreatic weight to body weight ratios (pw/bw) were calculated and are reported in mg/g.

Analysis of plasma samples: all blood samples were centrifuged at 2000 rpm for 30 min immediately after collection. Serum amylase levels were determined by means of the Phadebas test [3] and are reported in standard units. TNF was titrated in a bioassay on cell line

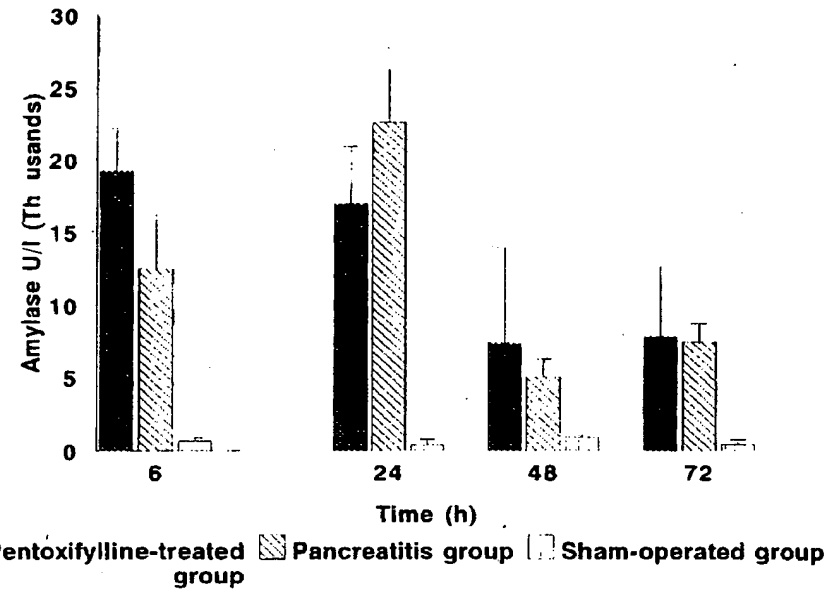

Fig. 1. Serum amylase activity in taurocholic acid (200 µl 6%)-induced acute pancreatitis in rats. The pentoxifylline-treated group (taurocholic acid intraductally + pentoxifylline intraperitoneally), the pancreatitis group (taurocholic acid alone) and the sham-operated group of animals were surgically prepared and killed as described in the Materials and methods section. Values are means ± SEM for groups of six rats

WEHI-164 [5]. IL-6 was measured via its proliferative action on the IL-6-dependent mouse hybridoma cell line B-9 [2]. The activities were calibrated against rm TNF (Genzyme, Cambridge, UK) and rm IL-6 (Sigma-Aldrich, Munich, Germany).

Histological examinations: the pancreas fragments were fixed in 10% neutral formaldehyde solution, embedded in paraffin and stained with hematoxylin and eosin, and Crossmon's trichrome for light microscopy. The different histologic lesions were scored in accordance with Hughes [11] on three rats at each time point.

Statistical analysis: results were expressed as means ± SEM. Statistical analysis was performed with the Statgraphics program (STSC, Statistical Graphics Corporation, two-sample analysis). *P*-values less than 0.05 were accepted as significant.

Results

The ratio pw/bw was not increased significantly after taurocholic acid injection. Pentoxifylline treatment did not change pw/bw significantly either. The increase in the serum amylase activity was significant in the pentoxifylline-treated group ($19.2\pm2.9\times10^3$ U/l and $16.9\pm3.9\times10^3$ U/l) and in the control group ($12.5\pm4.6\times10^3$ U/l and $22.5\pm5.1\times10^3$ U/l) as compared with the sham-operated group at 6 h and 24 h, but somewhat lower at 48 and 72 h (Fig. 1). There was no major difference in amylase level between the pancreatitis group and the pentoxifylline group. The histologic scores for the pancreatitis group and the pentoxifylline-treated group at various time points are shown in Table 1. The scoring results indicated that 48 h after the induction of pancreatitis the severity of the acinar necrosis is more evident in the pancreatitis group (score: 1.5 = lobular necrosis in 30% to 50% of the surface area, with micro-

Table 1. Scores of the different lesions in the pancreas according to the time of sacrifice in the pentoxifylline-treated group and the pancreatitis group. The different histologic lesions were scored in accordance with Hughes [11] on three rats at each time point

	Edema (0–1)	Vascular change (0–2)	Inflammation (0–1)	Acinar necrosis (0–2)	Fat necrosis (0–0.5)	Fibrosis (0–0.5)
Pancreatitis group (taurocholic acid)						
6 h	0.83	0.5	0.36	0.6	0	0
24 h	1.16	0.83	0.83	1.16	0	0
48 h	0.83	1.0	1.0	1.5	0	0.5
Taurocholic acid + pentoxifylline treatment						
6 h	0.83	0.6	0.36	1.16	0	0
24 h	1	0.83	0.83	1.3	0	0
48 h	0.8	1	1	0.66	0.5	0.5

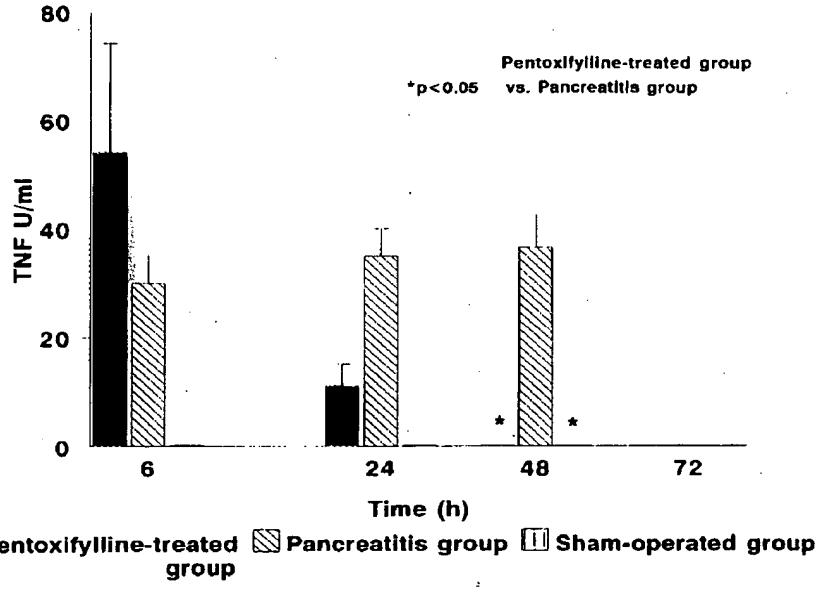

Fig. 2. Changes in serum TNF levels with time in taurocholic acid (200 µl 6%)-induced acute pancreatitis in rats. TNF was titrated in a bioassay on cell line WEHI-164. The activities were calibrated against rm TNF. Values are means ± SEM for groups of six rats

abscesses), while in the pentoxifylline-treated group at the same time point, focal acinar cell necrosis was detected with mild pancreatic inflammation (score: 0.66). TNF was not detected at any time in the sham-operated group. The TNF was elevated in the pentoxifylline-treated group (54.10± 20 U/ml and 10.9 ± 4.2 U/ml) at 6 h and 24 h, but was not detectable at 48 h. There was no detectable amount of TNF in any group at 72 h (Fig. 2). The IL-6 was elevated in the pentoxifylline-treated group at 6 h and 24 h (6463±1307 pg/ml, 10329±5571 pg/ml) but significantly decreased at 48 and 72 h (137.5±85.5 pg/ml and 71.4±188 pg/ml) as compared with the pancrea-

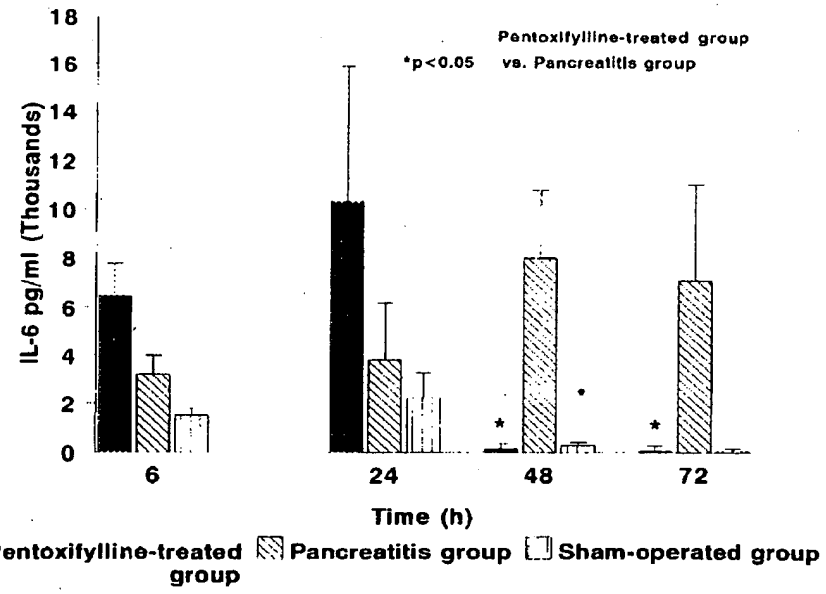

Fig. 3. Changes in serum IL-6 levels with time in taurocholic acid (200 µl 6%)-induced acute pancreatitis in rats. IL-6 was measured via its proliferative action on the IL-6-dependent mouse hybridoma cell line B-9. The activities were calibrated against rm IL-6. Values are means ± SEM for groups of six rats

titis group (8014±2793 pg/ml and 7083±2844 pg/ml, respectively) (Fig. 3). The mortality at 48 h was 43% in the pancreatitis group and 11% in the pentoxifylline-treated group.

Discussion

Despite new methods of surgical and intensive care, acute necrotizing pancreatitis remains a medical challenge. The high mortality and morbidity rates demand new efforts in the research on this pathologic condition. There is growing evidence that activation of the cytokine cascade is the key event in the necrosis of the pancreas and the development of extrapancreatic (systemic) complications. It is not easy to find an experimental model which is highly similar to acute necrotizing pancreatitis, but the taurocholic acid-induced model seems reasonably comparable to the human condition. Intraductal administration of 200 µl taurocholic acid (pancreatitis group) provoked severe acute necrotizing pancreatitis in rats. The pw/bw ratio did not change significantly in the pancreatitis group or in the pentoxifylline-treated group. The significant increases in the serum amylase level demonstrated that the pancreas was affected in both the pancreatitis group and the pentoxifylline-treated group. Pentoxifylline treatment did not decrease the serum amylase level relative to that in the control group. The histologic score differed in the pentoxifylline-treated group only slightly from that in the pancreatitis group after 48 h (acinar necrosis score 0.66 vs 1.5, respectively). These facts suggest that pentoxifylline does not appreciably influence the early events of taurocholic acid-induced

acute pancreatitis and the autodigestion of the pancreas. This seems reasonable because the early events of taurocholic acid-induced acute pancreatitis are produced by mechanical (increased intraductal pressure) and chemical destruction of the pancreas, and these processes may not be influenced by pentoxifylline or any other anticytokine treatment. The TNF level decreased rapidly after 6 h in the pentoxifylline-treated group. The lack of a detectable TNF level in the sham-operated group indicates that laparotomy itself is not as serious an injury as taurocholic acid-induced acute pancreatitis. The rapid decrease in IL-6 level in the pentoxifylline-treated group as compared with the pancreatitis group is very similar to the change in TNF.

These data suggest that activation of the cytokine cascade, and especially TNF, may play a pivotal role in the development of severe acute necrotizing pancreatitis. Pentoxifylline treatment is not able to protect the pancreas from acute damage (elevated serum amylase level, and elevated TNF and IL-6 in the early phase). The histologic results also indicate that pentoxifylline administration does not prevent the acute damage of the pancreas, but it can modify the severity of the inflammatory processes of the pancreas in this experimental model (at 48 h, the time when the TNF and the IL-6 levels were decreased). The lower mortality rate (11% in the pentoxifylline-treated group vs 43% in the pancreatitis group) also lends support to this concept. Via its blocking effect on TNF production and attenuation of the activation of the cytokine cascade, pentoxifylline may be beneficial in the complex treatment of acute necrotizing pancreatitis.

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AUTHOR ADDRESS: (a)Dep. Surg., UCLA Med. Cent., Los Angeles, CA**USA JOURNAL: Surgical Forum 48 (0):p179-180 1997
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Cell Biology

Soluble interleukin-6 receptor triggers osteoclast formation by interleukin 6

(bone resorption/gp130/interleukin-11/oncostatin M/leukemia inhibitory factor)

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ABSTRACT **It has been reported that soluble interleukin (IL)-6 receptor (sIL-6R) is detected in the serum of healthy individuals and its level is increased in patients with multiple myeloma and human immunodeficiency virus infection. Although several reports have suggested that sIL-6R potentiates IL-6 action, its physiological role remains unclear. In this study, we examined the role of sIL-6R on osteoclast formation by IL-6, using a coculture of mouse osteoblasts and bone marrow cells. Neither recombinant mouse IL-6 (mIL-6) nor mouse sIL-6R (smIL-6R) induced osteoclast-like multinucleated cell (MNC) formation when they were added separately. In contrast, simultaneous treatment with mIL-6 and smIL-6R strikingly induced MNC formation. These MNCs satisfied major criteria of authentic osteoclasts, such as tartrate-resistant acid phosphatase (TRAP) activity, calcitonin receptors, and pit formation on dentine slices. The MNC formation induced by mIL-6 and smIL-6R was dose-dependently inhibited by adding monoclonal anti-mouse IL-6R antibody (MR16-1). It is likely that osteoblasts and osteoclast progenitors are capable of transducing a signal from a complex of IL-6 and sIL-6R through gp130, even though they may have no or a very small number of IL-6Rs. Factors such as IL-11, oncostatin M, and leukemia inhibitory factor, which are known to exert their functions through gp130 (the signal-transducing chain of IL-6R), also induced MNC formation in our coculture system. These results suggest that increased circulating or locally produced sIL-6R induces osteoclast formation in the presence of IL-6 mediated by a mechanism involving gp130. This may play an important physiological or pathological role in conditions associated with increased osteoclastic bone resorption.**

Interleukin (IL)-6 is a multifunctional cytokine that regulates pleiotropic functions of cells and tissues (1). Several lines of evidence have suggested that IL-6 is an osteotropic factor as well. Löwik *et al.* (2) and we (3) independently reported that IL-6 stimulated *in vitro* bone resorption in fetal mouse metacarpals and calvaria, respectively. IL-6 is produced by both osteoblastic MC3T3-E1 cells and primary osteoblasts in response to IL-1, tumor necrosis factor α, and lipopolysaccharides (3). Littlewood *et al.* (4) also showed that human osteoblasts produced IL-6 in response to several external stimuli. Besides these *in vitro* studies, IL-6 has been reported to stimulate osteoclast formation and bone resorption *in vivo* as well. Yoneda *et al.* (5) showed that transplantation of human tumor (MH-85) cells into *nude* mice, in which these cells produce a high level of human IL-6, caused hypercalcemia and that administration of neutralizing anti-IL-6 antibody to these tumor-bearing mice lowered the serum calcium to a normal level. Similar hypercalcemic action of IL-6 was reported by Black *et al.* (6), who used Chinese hamster ovarian (CHO) cells transfected with the IL-6 gene. More recently, Jilka *et al.* (7) reported that IL-6 may contribute to bone loss caused by estrogen deficiency. They showed that estrogen inhibited IL-6 production by osteoblasts *in vitro* (8) and that anti-IL-6 antibody prevented osteoclast development caused by estrogen deficiency *in vivo* (7). Taken together, these data suggest that IL-6 plays an important role in stimulating bone resorption in some physiological and/or pathological conditions. However, the mechanism of action of IL-6 responsible for osteoclast recruitment and bone resorption *in vitro* has not been established.

We have developed a coculture system of mouse primary osteoblastic cells and bone marrow cells (or spleen cells) to assess the effects of hormones and cytokines on osteoclast formation (9, 10). In this system, osteoclast-like multinucleated cells (MNCs), which satisfied major criteria of authentic osteoclasts, were formed in response to several osteotropic factors, such as 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$] (9, 10), parathyroid hormone (9, 11) and parathyroid hormone-related peptides (12), prostaglandins (13), and IL-1 (14). To date, however, we have failed to demonstrate stimulative effects of IL-6 on MNC formation in our coculture system. This led us to speculate that increased production of IL-6 may not be enough to induce osteoclast formation.

IL-6 exerts its activity via a cell surface receptor which consists of two components: a ligand-binding 80-kDa glycoprotein chain (IL-6R) and a non-ligand-binding but signal-transducing 130-kDa glycoprotein chain (gp130) (15). When IL-6R is occupied by IL-6, IL-6R is associated with gp130, then mediates the IL-6 functions. More recently, it was reported that the genetically engineered human and mouse soluble IL-6R (sIL-6R), which lacks transmembrane and cytoplasmic regions, could also mediate the IL-6 signal through gp130 (15–18). Native sIL-6R has been detected in the urine (19) and sera of healthy subjects (20), and its level is increased in patients with multiple myeloma (21) and human immunodeficiency virus infection (20). Suzuki *et al.* (22) reported that serum sIL-6R levels in MRL/*lpr* mice, an animal model of autoimmune diseases, were increased with age.

In this study, we examined the role of mouse sIL-6R (smIL-6R) in MNC formation in the presence of mouse IL-6

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Abbreviations: IL, interleukin; IL-6R, IL-6 receptor; sIL-6R, soluble IL-6R; smIL-6R, mouse sIL-6R; mIL-6, mouse IL-6; mIL-6R, mIL-6 receptor 1α,25$(OH)_2D_3$ or 1,25D_3, 1α,25-dihydroxyvitamin D_3; MNC, multinucleated cell; CT, calcitonin; OSM, oncostatin M; LIF, leukemia inhibitory factor; FBS, fetal bovine serum; TRAP, tartrate-resistant acid phosphatase.

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(mIL-6) in our coculture system of mouse osteoblastic cells and bone marrow cells. The results presented here indicate that smIL-6R triggers MNC formation by mIL-6, suggesting that locally or systemically produced sIL-6R is involved in IL-6-mediated osteoclast recruitment and osteoclastic bone resorption.

MATERIALS AND METHODS

Animals and Drugs. Female Wistar rats and BALB/c *nu/nu* mice were purchased from Charles River Japan (Kanagawa). Newborn ddY mice and 6- to 9-week-old male ddY mice were obtained from Shizuoka Laboratories Animal Center (Shizuoka, Japan). $1\alpha,25(OH)_2D_3$ was purchased from Duphar (Weesp, The Netherlands). Recombinant human interleukin 1α (IL-1) was purchased from Genzyme. Synthetic salmon calcitonin (CT) was purchased from Peninsula Laboratories. Recombinant human IL-11, human oncostatin M (OSM), and human leukemia inhibitory factor (LIF) were purchased from Pepro Tech (Rocky Hill, NJ). Collagen gel solutions (Cellmatrix, type I-A) were obtained from Nitta Gelatin Co. (Osaka). Bacterial collagenase was obtained from Wako Pure Chemical (Osaka). Dentine (ivory) was kindly provided by Nishide Inzai Co. (Tokyo). Other chemicals and reagents used in this study were of analytical grade.

Preparation of Recombinant mIL-6. The mIL-6-producing CHO cells were established by transfection of dihydrofolate reductase-deficient CHO cells with an mIL-6 cDNA expression vector. The mIL-6 cDNA was isolated from P388D1 (IL-1) cells by PCR cloning. A high producing clone was selected by amplifying with 50 nM methotrexate. The established clone was cultured in α minimal essential medium (α-MEM) containing 5% fetal bovine serum (FBS) at 37°C in a humidified atmosphere of 5% CO_2 in air. The culture media were collected and used as mIL-6 preparations in the following studies. Concentrations of mIL-6 in the media were determined by a sandwich ELISA using monoclonal anti-mIL-6 antibody 6B4 (kindly provided by J. Van Snick, Ludwig Institute for Cancer Research, Brussels), and rabbit polyclonal anti-mIL-6 antibody.

Preparation of Recombinant smIL-6R. The smIL-6R-producing CHO cells were prepared as described (17). The cells were cultured in α-MEM containing 5% FBS at 37°C in a humidified atmosphere of 5% CO_2 in air. Conditioned media were collected and used as smIL-6R preparations in the following studies. Concentrations of smIL-6R in the media were determined by a sandwich ELISA using rat monoclonal anti-mIL-6 receptor (mIL-6R) antibody RS15 (17) and rabbit polyclonal anti-mIL-6R antibody.

Preparation of Rat Monoclonal Anti-mIL-6R Antibody MR16-1. smIL-6R was purified from smIL-6R preparations with an affinity column-adsorbed rat monoclonal anti-mIL-6R antibody (RS12) (17). Wistar rats were immunized with 50 μg of purified smIL-6R in Freund's complete adjuvant (s.c.), followed 2 weeks later by four 50-μg boosts of smIL-6R in Freund's incomplete adjuvant once a week (s.c.). One week after the last boost, the rats were injected with 50 μg of smIL-6R in 100 μl of phosphate-buffered saline (PBS) (i.v.). The rats were sacrificed 3 days later, and spleen cells were fused with mouse P3U1 myeloma cells at a ratio of 10:1 with polyethylene glycol 1500 (Boehringer Mannheim). After overnight incubation at 37°C in the wells of a 96-well plate (Falcon 3075) with 100 μl of complete RPMI 1640 medium containing 10% FBS, 100 μl of hypoxanthine/aminopterin/thymidine (HAT) containing human IL-6 (23) was added to each well. Half of the culture medium was replaced with HAT medium daily for 4 days. Seven days later, hybridomas producing anti-smIL-6R were selected by a smIL-6R-binding assay (ELISA). Briefly, culture supernatants of the hybridomas (100 μl) were incubated for 60 min in plates coated with rabbit polyclonal anti-rat IgG antibody (Cappel) at 1 μg/ml. The plates were washed, and incubated with smIL-6R at 100 ng/ml. After washing, rabbit polyclonal anti-mIL-6R antibody was added at 2 μg/ml, and the plates were washed and incubated with alkaline phosphatase-conjugated goat polyclonal anti-rabbit IgG antibody (Tago) for 60 min. Finally, after washing, the plates were incubated with a substrate (Sigma 104; *P*-nitrophenyl phosphate) of alkaline phosphatase for 30 min, and were read by using a plate reader (Tosoh, Tokyo) at 405 nm. Hybridomas recognizing smIL-6R were cloned twice by limiting dilution. For ascites production, BALB/c *nu/nu* mice were injected twice with 0.5 ml of pristane, and 3 days later, 3×10^6 established hybridoma cells were injected (i.p.). Ascites fluid was collected 10–20 days later, and the monoclonal antibody (MR16-1) was purified from the fluid with protein G column (Oncogene Science).

Coculture of Osteoblastic Cells and Bone Marrow Cells. Coculture of osteoblastic cells and bone marrow cells was performed by the method of Akatsu *et al.* (10) using *ddy* mice. In short, primary osteoblast-like cells (1×10^4 per well) obtained from mouse calvaria and nucleated marrow cells (2×10^5 per well) were cocultured in the wells of a 48-well plate (Sumitomo Bakelite, Tokyo) with 0.3 ml of α-MEM containing 10% FBS in the presence of test chemicals. Cultures were performed in quadruplicate and cells were fed on day 4 by replacing 0.25 ml of old medium with fresh medium. To determine bone-resorbing activity of osteoclast-like MNCs formed, we used MNC preparations as described (10). In short, primary osteoblast-like cells (5×10^5 per dish) and nucleated marrow cells (6×10^6 per dish) were cocultured in the presence of mIL-6 at 200 ng/ml and smIL-6R at 500 ng/ml on collagen-gel coated dishes. After culturing for 6–7 days, dishes were treated with 4 ml of 0.2% bacterial collagenase for 20 min. The recovered cell suspensions were gently layered on 35% Percoll solution and centrifuged at $250 \times g$ for 20 min. The cells which accumulated at the interface were collected as MNC preparations, suspended in α-MEM containing 10% FBS, and used for determining the activity of pit formation on dentine slices.

Determination of Osteoclast Characteristics. MNCs were fixed and stained for tartrate-resistant acid phosphatase (TRAP), and the number of TRAP-positive MNCs was counted (10). Pit formation was assessed as described (24). Briefly, MNC preparations were put on dentine slices (diameter 4 mm, ≈200 μm thick) in the wells of a 96-well plate (Corning 25820). The slices were then incubated in humidified atmosphere at 37°C in α-MEM containing 10% FBS with or without CT. After they had been cultured for 48 h, resorption pits were visualized with Mayer's hematoxylin staining, and the areas of image of Mayer's hematoxylin were measured with a personal image analysis system (LA-525, PIAS Co., Tokyo) linked to a light microscope. For autoradiography using ^{125}I-labeled salmon CT (^{125}I-CT), cocultures were performed on coverslips placed in 24-well plates. Cultures were then incubated with 2×10^{-10} M ^{125}I-CT, stained for TRAP, and processed for autoradiography as described (10).

Statistical Analysis. Data are expressed as mean ± SEM. The statistical significance of the differences between the control and the experimental group was determined by Student's *t* test.

RESULTS

MNC Formation by mIL-6 and smIL-6R. Fig. 1 shows the effects of mIL-6 and smIL-6R on MNC formation when they were separately applied to the cocultures. $1\alpha,25(OH)_2D_3$ (10^{-8} M) was used as a positive control stimulator of MNC formation. Neither mIL-6 (0.2–200 ng/ml) nor smIL-6R (0.5–50 ng/ml) stimulated MNC formation. smIL-6R at 500

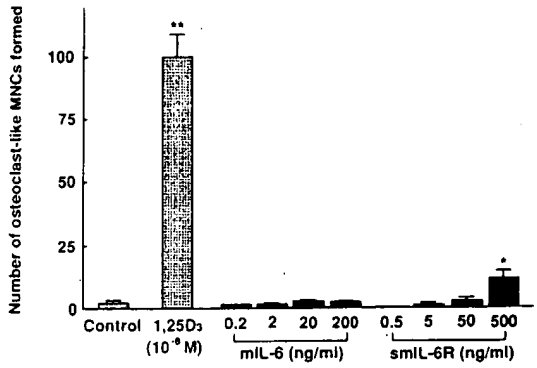

FIG. 1. Effects of mIL-6 or smIL-6R alone on the MNC formation in cocultures of osteoblastic cells and bone marrow cells. Mouse primary osteoblastic cells and bone marrow cells were cocultured with graded concentrations of either mIL-6 or smIL-6R. $1\alpha,25(OH)_2D_3$ ($1,25D_3$, 10^{-8} M) was used as a positive control stimulator of MNC formation. After culture for 7 days, TRAP-positive MNCs formed were counted. Data are expressed as the mean ± SEM of quadruplicate cultures. Significantly different from the control cultures: *, $P < 0.05$; **, $P < 0.01$.

ng/ml induced a small but significant increase in MNC formation. To investigate the possibility that smIL-6R potentiates the mIL-6 action on MNC formation, mIL-6 and smIL-6R were simultaneously added to the cocultures (Fig. 2). mIL-6 and smIL-6R cooperatively stimulated MNC formation, and the potency was increased dose dependently. Minimum concentrations required to induce significant MNC formation were 2 ng/ml of mIL-6 and 62.5 ng/ml of smIL-6R (Fig. 2). These results suggest that coexistence of smIL-6R and mIL-6 is essential for stimulating the MNC formation by IL-6. Since sIL-6R alone fails to transduce the IL-6 signal (15), the MNC formation induced by the maximum dose (500 ng/ml) of smIL-6R alone (Fig. 1) appears to result from the presence of endogenous mIL-6 produced by osteoblastic cells in our cocultures (3).

Effects of Monoclonal Anti-mIL-6R Antibody on MNC Formation. To verify that the MNC formation shown in Fig. 2 was due to the formation of a complex of mIL-6 and smIL-6R, we examined the effects of rat monoclonal anti-mIL-6R antibody, MR16-1, on MNC formation. Adding

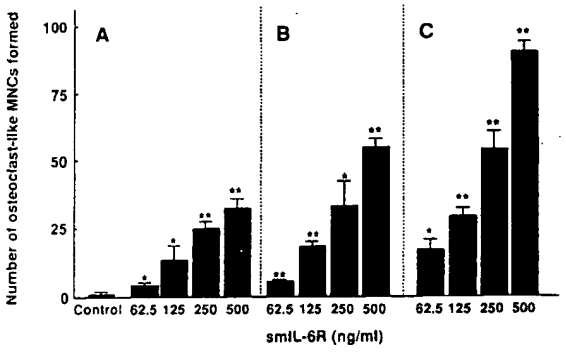

FIG. 2. Cooperative effects of mIL-6 and smIL-6R on the MNC formation in cocultures of osteoblastic cells and bone marrow cells. Mouse primary osteoblastic cells and bone marrow cells were cocultured with mIL-6 at 2 ng/ml (*A*), 20 ng/ml (*B*), or 200 ng/ml (*C*) in the presence of graded concentrations of smIL-6R. After being cultured for 7 days, TRAP-positive MNCs formed were counted. Data are expressed as the mean ± SEM of quadruplicate cultures. Significantly different from the control cultures: *, $P < 0.05$; **, $P < 0.01$.

graded concentrations of MR16-1 dose-dependently decreased MNC formation induced by mIL-6 (20 ng/ml) and smIL-6R (500 ng/ml) (Fig. 3*A*). In contrast, as shown in Fig. 3*B*, MR16-1 at 100 ng/ml had no effect on MNC formation induced by $1\alpha,25(OH)_2D_3$ or IL-1. Control IgG had no inhibitory effect on MNC formation induced by mIL-6 and smIL-6R (data not shown).

Determination of Osteoclast Characteristics of MNCs. To determine the ability to form resorption pits, MNC preparations induced by mIL-6 and smIL-6R were isolated from collagen-gel-coated dishes (10) and cultured for 48 h on dentine slices. MNCs formed many resorption pits on dentine slices (Fig. 4*A*). The resorbing activity of the MNCs expressed by the plan area resorbed per MNC was 4600 ± 400 μm^2, which was comparable to that of $1\alpha,25(OH)_2D_3$-induced MNCs (4100 ± 400 μm^2) (data not shown). When 10^{-11} M salmon CT was added, the ability to form resorption pits of MNCs induced by mIL-6 and smIL-6R was completely inhibited (Fig. 4*B*). Autoradiographic studies using ^{125}I-CT indicated that numerous dense grains due to the ^{125}I-CT binding appeared on more than 95% of the TRAP-positive MNCs (Fig. 4*C*).

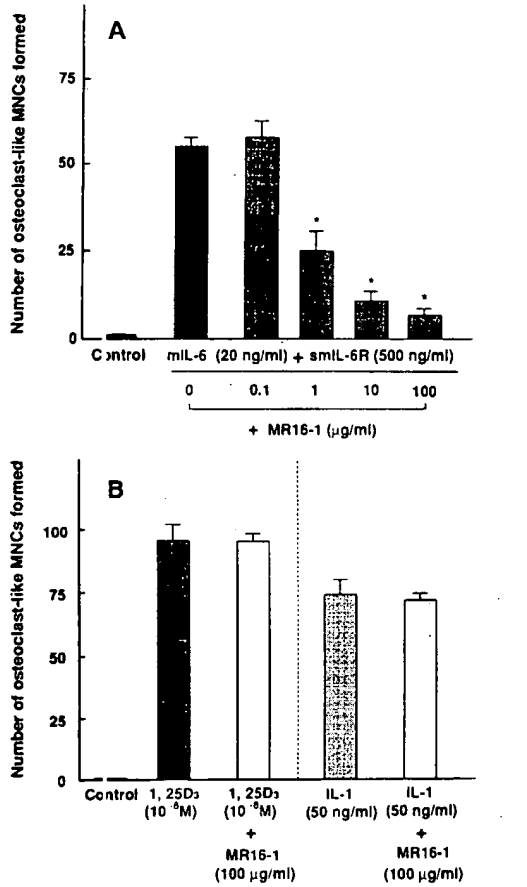

FIG. 3. Effect of monoclonal anti-mIL-6R antibody on MNC formation. Mouse primary osteoblastic cells and bone marrow cells were cocultured with mIL-6 (20 ng/ml) and smIL-6R (500 ng/ml) (*A*), or $1\alpha,25(OH)_2D_3$ (10^{-8} M) or IL-1 (50 ng/ml) (*B*), both with or without rat monoclonal anti-mIL-6R antibody, MR16-1. Graded concentrations (*A*) or 100 μg/ml (*B*) of MR16-1 was added at the beginning of the cocultures. After culture for 7 days, TRAP-positive MNCs formed were counted. Data are expressed as the mean ± SEM of quadruplicate cultures. Significantly different from the cultures treated with mIL-6 and smIL-6R alone: *, $P < 0.01$.

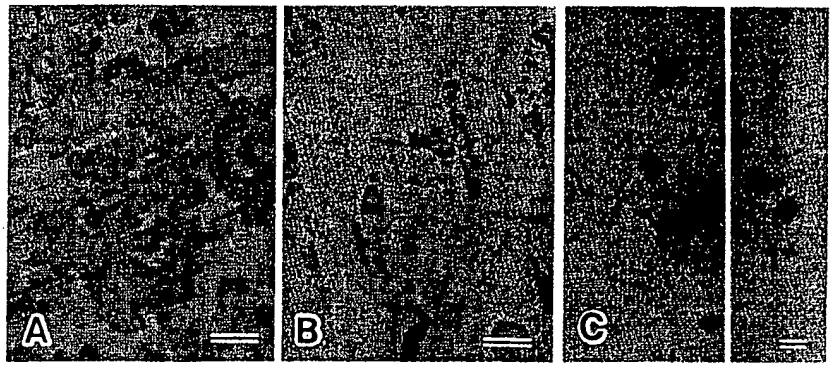

FIG. 4. Osteoclast characteristics of MNCs induced by mIL-6 and smIL-6R. Pit formation on dentine slices by MNCs was examined in the absence (*A*) or presence (*B*) of salmon CT (10^{-11} M) at 37°C for 48 h. Resorption pits were stained with Mayer's hematoxylin and observed under a light microscope. Localization of TRAP activity and binding of ^{125}I-labeled salmon CT in MNCs were assessed as described in the text (*C*). (Bars = 100 μm.)

Effects of Cytokines Which Utilize gp130 as a Common Signal Transducer on MNC Formation. Recent studies have revealed that not only IL-6 but also several cytokines transduce their signals through gp130 (25, 26). To confirm that the signal mediated by gp130 is involved in MNC formation, we examined the effects of IL-11, OSM, and LIF, all of which transduce their signals through gp130, on MNC formation. All the factors tested induced MNC formation dose dependently (Fig. 5). The potency of these factors in inducing MNCs was the highest in OSM, followed by IL-11 and LIF, in that order.

DISCUSSION

The present study clearly demonstrates that smIL-6R triggers formation of osteoclast-like MNCs in the presence of mIL-6, but mIL-6 alone does not. MNCs induced by mIL-6 and smIL-6R satisfied major criteria of authentic osteoclasts, such as TRAP activity, functional CT receptors, and pit formation on dentine slices (Fig. 4). This indicates that the MNCs induced by mIL-6 and smIL-6R are indeed genuine osteoclasts. It has been reported that sIL-6R potentiates agonistic effects in the presence of IL-6 (15–18, 21, 22). However, most of the results so far reported have been obtained by using IL-6-dependent cell lines or IL-6R or gp130 gene-transfected cells. We now add MNCs formed in the cocultures of osteoblastic cells and bone marrow cells to the list of cells which require sIL-6R in the action of IL-6. mIL-6 induced MNC formation in the presence of smIL-6R, but either of the two alone did not. Therefore, our coculture system appears to provide useful information to elucidate the role of sIL-6R.

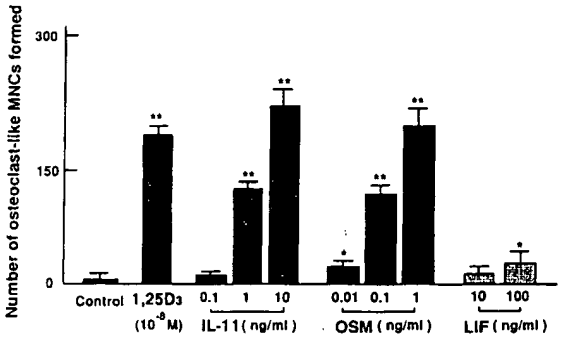

FIG. 5. Effects of several cytokines that utilize gp130 as a common signal transducer on MNC formation. Mouse primary osteoblastic cells and bone marrow cells were cocultured with graded concentrations of IL-11 (0.1–10 ng/ml), OSM (0.01–10 ng/ml), or LIF (10 and 100 ng/ml). $1\alpha,25(OH)_2D_3$ (10^{-8} M) was used as a positive control stimulator of MNC formation. After culture for 7 days, TRAP-positive MNCs formed were counted. Data are expressed as the mean ± SEM of quadruplicate cultures. Significantly different from the control cultures: *, $P < 0.05$; **, $P < 0.01$.

There is very little information on the distribution of membrane-bound IL-6R on osteoblasts and bone marrow cells. Littlewood *et al.* (4) reported that IL-6R mRNA was detected in human osteoblast-like cells, but IL-6 showed no effects on these cells. Both their report and the present study suggest that osteoblastic cells and/or osteoclast progenitor cells present in bone marrow cells have no or a very small number of functional IL-6Rs in physiological conditions. Since the gp130 gene has been reported to be expressed ubiquitously (1), these cells may interact with a complex of mIL-6 and smIL-6R and mediate the IL-6 signal via gp130 even in the absence of IL-6R. Recent studies have revealed that several cytokines mediate their respective signals via gp130 as IL-6 does (25, 26). Passeri *et al.* (27) have reported that IL-11, which also transduces its signal through gp130 (26), stimulates osteoclast formation and osteoclastic bone resorption *in vitro*. In the present study, we examined the effects of three cytokines on MNC formation, OSM, IL-11, and LIF, all of which utilize gp130 as a signal transducer (25, 26). All the cytokines induced MNC formation (Fig. 5). Of the three cytokines tested, OSM and IL-11 were potent MNC inducers. LIF also induced MNC formation, but its potency was much less than that of OSM and IL-11. These cytokines exhibit redundancy (overlapping functions), but each factor also possesses its own specific activities (25). This may be explained by the specific distribution of their respective receptors in the target cells (25). Therefore, the potency of these cytokines to induce MNCs may reflect the receptor number of each cytokine in osteoblastic cells and/or bone marrow cells.

On the basis of these results, gp130 appears to be involved in MNC formation induced by IL-6, OSM, IL-11, and LIF. We have reported that MNC formation is induced by at least two different mechanisms; one is parathyroid hormone-, IL-1-, and prostaglandin E_2-induced MNC formation, which is mediated by a mechanism involving cAMP production, and the other is $1\alpha,25(OH)_2D_3$-induced MNC formation, the mechanism of which appears to be independent of cAMP production (28). IL-1 induces MNC formation by a mechanism involving prostaglandin production (14). In both mechanisms, osteotropic factors appear to act directly on osteoblastic cells, which in turn produce a factor responsible for osteoclast differentiation (28). Such a factor appears to be expressed on the cell surface membranes and plays a key role through a cell-to-cell contact mechanism (28). In the present study, we showed that anti-mIL-6R antibody inhibited mIL-6- and smIL-6R-induced MNC formation but not IL-1- or $1\alpha,25(OH)_2D_3$-induced MNC formation (Fig. 3). Therefore, IL-6 production does not appear to be involved in the MNC formation by IL-1 or $1\alpha,25(OH)_2D_3$-mediated signals,

respectively. A preliminary experiment showed that, when osteoblastic cells and spleen cells (osteoclast progenitor cells) were cocultured without direct contact, no MNCs were formed in response to smIL-6R and mIL-6, IL-11, or OSM (data not shown). This indicates that a microenvironment provided by osteoblastic cells or cell-to-cell contact between osteoblastic cells and osteoclast progenitor cells is indispensable for gp130-mediated MNC formation. Further studies are necessary to clarify how the gp130-mediated signal stimulates osteoclast formation.

Recent studies have revealed that naturally produced sIL-6R occurs in the urine (19) and sera (20) of healthy subjects, and that its serum levels are increased in patients with multiple myeloma (21), human immunodeficiency virus infection (20). Furthermore, excess IL-6 is produced systemically and locally in patients with multiple myeloma (29, 30). Both IL-6 and sIL-6R levels were also significantly elevated in synovial fluids from patients with rheumatoid arthritis (31, 36). The results of the present study taken together with these results suggest that the elevated levels of both IL-6 and sIL-6R form a complex, which is involved in bone destruction and induces hypercalcemia in these patients. Recently Jilka *et al.* (7) have proposed that IL-6 is responsible for osteoclast recruitment caused by estrogen deficiency. They reported that the increase in osteoclast number after ovariectomy was prevented by the treatment with a neutralizing anti-IL-6 antibody *in vivo* and in *ex vivo* cultures (7). Interestingly, anti-IL-6 antibody had no inhibitory effects on osteoclastogenesis in sham-operated animals (7). Costatini *et al.* (32) reported that IL-6 gene knock-out mice did not lose bone after ovariectomy. This confirms the previous report that IL-6 is involved in bone resorption in estrogen deficiency. We also speculate that estrogen deficiency may cause excess production of not only IL-6 but also (s)IL-6R in bone cells. Further studies are needed to prove these hypotheses.

There have been many reports which suggest that IL-6 plays an important role in osteoclastic bone resorption *in vitro* as well as *in vivo* (33). However, several conflicting results have been reported. Al-Humidan *et al.* (34) and Barton and Mayer (35) failed to demonstrate bone-resorbing activity of IL-6 on neonatal calvariae *in vitro*. Al-Humidan *et al.* (34) also showed that IL-6 might act as a local inhibitor of bone resorption. In addition, Löwik *et al.* (2) reported that IL-6 stimulated bone resorption in metacarpals but not in fetal long bones in organ cultures. The discrepancy may be explained by the differences in experimental conditions or cellular composition of bone tissues used, or species differences, but a clear explanation remains to be found. Our results raise the possibility that the number of IL-6Rs on the cell membrane or the concentration of sIL-6R in assay systems may be important factors controlling IL-6 response in target tissues in physiological and/or pathological conditions.

In conclusion, sIL-6R triggers osteoclast formation in the presence of IL-6. Excess production of IL-6 and (s)IL-6R may contribute to osteoclastic bone resorption in several metabolic bone diseases, such as multiple myeloma, rheumatoid arthritis, and postmenopausal osteoporosis. If this hypothesis is correct, anti-IL-6R antibody will be useful as an inhibitor of sIL-6R and IL-6R for the treatment of metabolic bone diseases.

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Characterization of anti-mouse interleukin-6 receptor *antibody*. Okazaki Makoto; Yamada Yoshiki; Nishimoto Norihiro; Yoshizaki Kazuyuki; Mihara Masahiko
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103 Method Claim

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Farkas G; Marton J; Nagy Z; Mandi Y; Takacs T; Deli M A; Abraham C S Department of Surgery, Albert Szent-Gyorgyi University Medical School, Szeged, Hungary. farkas@surg.szote.u-szeged.hu
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AUTHOR ADDRESS: (a)Dep. Surg., UCLA Med. Cent., Los Angeles, CA**USA JOURNAL: Surgical Forum 48 (0):p179-180 1997
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BLOCKAGE OF INTERLEUKIN-6 RECEPTOR AMELIORATES JOINT DISEASE IN MURINE COLLAGEN-INDUCED ARTHRITIS

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Objective. **To clarify the role of interleukin-6 (IL-6) in the pathogenesis of collagen-induced arthritis (CIA).**

Methods. **CIA was induced by immunizing twice at a 3-week interval with bovine type II collagen (CII) emulsified with complete adjuvant. Rat anti-mouse IL-6 receptor (anti–IL-6R) monoclonal antibody MR16-1 or isotype-matched control antibody KH-5 was then injected once intraperitoneally. Symptoms of arthritis were evaluated with a visual scoring system, and serum anti-CII antibody and IL-6 levels were measured by enzyme-linked immunosorbent assay. In addition, the CII responsiveness of splenic lymphocytes from mice with CIA was examined.**

Results. **In mice with CIA, excess production of IL-6 in sera was observed within 24 hours after the first CII immunization, and then rapidly decreased. Serum IL-6 increased again beginning 14 days after immunization, in conjunction with the onset of arthritis. When MR16-1 was injected immediately after immunization with CII, it inhibited the development of arthritis in a dose-dependent manner. Furthermore, MR16-1–treated mice exhibited lower serum levels of IgG anti-CII antibody and reduced responsiveness of lymphocytes to CII. This suppressive effect was observed when MR16-1 was injected on day 0 or 3, but not when injected on day 7 or 14.**

Conclusion. **IL-6 produced after CII immunization appears to play an essential role in the immunity to CII, and anti–IL-6R antibody reduces the development of CIA by suppressing IL-6 signal transduction.**

Collagen-induced arthritis (CIA) is an experimental autoimmune disease model that is widely used for studying disease processes in, and potential therapies for, human rheumatoid arthritis. CIA develops in rodents after immunization with type II collagen (CII) in adjuvant (1,2). Many investigators have shown that cytokines play essential roles in the pathogenesis of CIA. In particular, inflammatory cytokines, such as interleukin-1 (IL-1) and tumor necrosis factor α (TNFα), are considered to be key cytokines, on the basis of the finding that anti–IL-1 and anti-TNFα therapies suppress the development of CIA (3,4).

IL-6 exhibits a variety of biologic actions on the immune, central nervous, and hematopoietic systems (5). Furthermore, IL-6 can be produced by many kinds of cells after IL-1 and TNFα stimulation, and it induces the formation of acute-phase proteins by liver cells (5), suggesting that it participates strongly in the inflammatory response. Excess production of IL-6 has been observed in sera from animals with CIA (6,7). These lines of evidence introduced the possibility that IL-6 is involved in the pathogenesis of CIA; however, there was no direct evidence for IL-6 involvement in CIA development.

In the present study, to investigate IL-6 involvement in arthritis, we examined the effect of rat anti-mouse IL-6 receptor (IL-6R) monoclonal antibody (mAb) (MR16-1) in the murine CIA model. Our findings clearly demonstrated that MR16-1 significantly suppresses CIA onset, and that IL-6 is strongly involved in the pathogenesis of CIA.

MATERIALS AND METHODS

Animals. Eight-week-old male DBA/1J mice were purchased from Charles River Japan (Yokohama, Japan). The

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mice were specific pathogen free, and were kept in cages in a room maintained at 24°C (±2°C) and 50–60% relative humidity.

Monoclonal antibodies. Hybridoma MR16-1 cells (6), which produce anti-mouse IL-6R mAb (rat IgG1), and hybridoma KH-5 cells, which produce anti-dinitrophenol mAb (rat IgG1), were prepared in our laboratories. Cells were injected intraperitoneally into BALB/c nude mice that had been pretreated with pristane (Aldrich Chemical, Milwaukee, WI). Ascites fluid was collected, and the IgG fraction was obtained using a protein G column. Lipopolysaccharide (LPS) contamination of these antibody preparations was <20 pg/mg IgG.

Induction and clinical assessment of CIA. Bovine CII (Cosmo-Bio, Tokyo, Japan) was dissolved overnight at 4°C in 0.1*M* acetic acid solution, to a concentration of 4 mg/ml. At the age of 9 weeks, mice were immunized by intradermal injection at the body–tail junction with 200 μg of CII emulsified with an equal volume of complete adjuvant H37Ra (Difco, Detroit, MI). Three weeks later, the mice were boosted in the same manner.

The clinical symptoms of arthritis in all 4 limbs were evaluated with a visual scoring system. Arthritic lesions were graded on a scale of 0–4, where 0 = no change, 0.5 = swelling and erythema of 1 digit, 1 = swelling and erythema of 2 or more digits, 2 = mild swelling and erythema of the limb, 3 = gross swelling and erythema of the limb, and 4 = gross deformity and inability to use the limb. The arthritis score for each mouse was the sum of the score of each of the 4 limbs, the maximum possible score thus being 16.

Experimental protocol. Immunization with CII was performed as described above, and blood from the orbital vein was obtained by means of a capillary tube on day 1, 3, 7, 14, or 28 after immunization (n = 5 on each day). Blood was drawn only once from each mouse, to avoid production of IL-6 due to stress.

In experiment 1, MR16-1 at a dose of 0.5 mg, 2 mg, or 8 mg (n = 5 in each group) or isotype-matched rat IgG (KH-5) at a dose of 8 mg (n = 5) was injected intraperitoneally once on the day of the first collagen immunization (day 0). As a control, vehicle (pyrogen-free saline) was injected in the same manner (n = 5). Blood from the orbital vein was obtained via a capillary tube on day 34.

In experiment 2, MR16-1 at a dose of 8 mg was injected intraperitoneally once on day 0, 3, 7, 14, or 21 (n = 5 on each day). As a control, vehicle was injected on day 0 (n = 5).

Measurement of anti-CII antibody. Anti-CII antibody levels in sera were assessed by enzyme-linked immunosorbent assay (ELISA). Briefly, diluted sample serum was added to each well of bovine CII (5 μg/ml)–precoated immunoplates (MaxiSorp; Nunc, Roskilde, Denmark). After incubation for 2 hours at room temperature, bound IgG was measured using peroxidase-conjugated antibody to mouse IgG (Jackson ImmunoResearch, West Grove, PA). Substrate (*o*-phenylenediamine) was added, the calorimetric reaction was stopped with 6*N* H_2SO_4 solution, and measurement was carried out with a microplate reader (model 3550; Bio-Rad, Hercules, CA) at 490–550 nm. To convert optical density values to IgG concentrations, a standard curve was generated for each assay, using pooled CII-hyperimmunized mouse serum (defined as 1,000 units/ml).

T cell response to CII. Twenty-eight days after the first immunization with CII, spleens from 5 arthritic mice were pooled and dispersed as single cells in RPMI 1640 medium (Gibco, Grand Island, NY) containing 100 μg/ml streptomycin (Meiji Seika, Tokyo, Japan), 100 units/ml penicillin G (Meiji Seika), and 1% fetal bovine serum (Hyclone, Logan, UT). Erythrocytes were removed by centrifugation on Ficoll (Lympholite-M; Cedarlane, Hornby, Ontario, Canada). Mononuclear cells (4×10^5 cells/well/0.2 ml) were cultured with 12.5 μg/ml of heat-denatured CII (56°C, 10 minutes) or 1 μg/ml of phytohemagglutinin (PHA; Wellcome Foundation, Dartford, UK), in 96-well flat-bottomed microtiter plates (Falcon 3072; Becton Dickinson Labware, Lincoln Park, NJ), for 72 hours and 48 hours, respectively. Cultures were performed in triplicate. Tritiated thymidine (^{3}H-TdR, 1 μCi/well; Amersham, Buckinghamshire, UK) was added to each well for the last 12 hours of culture, and ^{3}H-TdR incorporation in the cells was measured using a liquid scintillation counter.

Measurement of serum IL-6. IL-6 concentrations in the sera were measured with the IL-6 ELISA kit, according to the manufacturer's instructions (Genzyme, Cambridge, MA). To measure IL-6 activity in the sera in duplicate cultures, cells from an IL-6–dependent cell line, MH60, were cultured in 96-well flat-bottomed culture plates (5×10^4 cells/well/0.2 ml), with serial 20-fold dilutions of samples. Forty hours later the cells were pulsed for 4 hours with ^{3}H-TdR (1 μCi/well). The cells were harvested onto glass filters, and the incorporated radioactivity was measured with a micro-beta plate reader.

Statistical analysis. Values are reported as the mean ± SEM. The statistical significance of differences was analyzed using the nonparametric Dunnett's multiple comparison test for the arthritis score and the parametric Dunnett's multiple comparison test for anti-CII antibody levels and the splenic lymphocyte proliferative response.

RESULTS

Serum IL-6 concentrations. On days 1, 3, 7, 14, and 28, we examined serum IL-6 levels in mice with CIA, by ELISA. Significant increases in serum IL-6 levels (>300 pg/ml) were detected on day 1, but they rapidly decreased, and disappeared by day 7. On day 28, increased serum IL-6 levels were observed again (Figure 1). The biologic activity of IL-6 was confirmed using the IL-6–dependent cell line MH60 (data not shown).

Arthritis development. Based on the above result, MR16-1 (in various doses) and KH-5 were administered intraperitoneally on the day of the first immunization with CII (experiment 1). In the vehicle- and KH-5–treated groups, all mice developed signs of arthritis within 31 days after the first immunization. The mean (±SEM) day of arthritis onset was day 27.0 ± 1.0 and day 28.6 ± 1.5 in the vehicle- and KH-5–treated groups, respectively, and the maximum arthritis score was 10.4 ± 1.7 and 10.8 ± 1.7, respectively. In contrast, the development of arthritis in the MR16-1–treated mice was

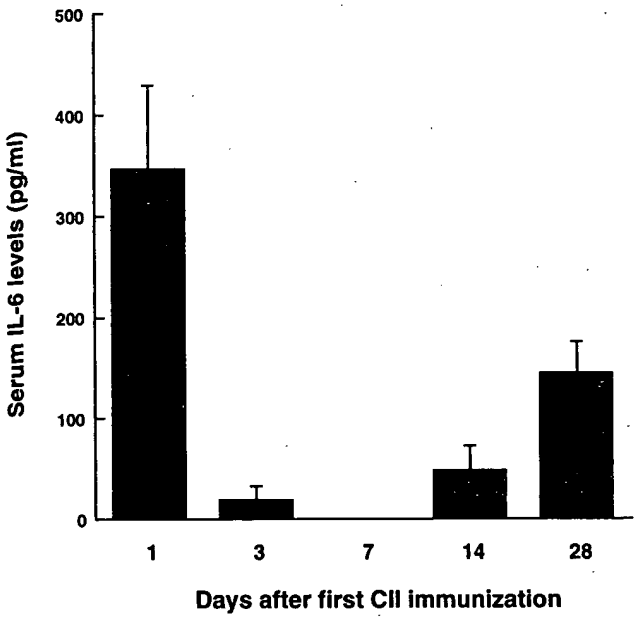

Figure 1. Serum interleukin-6 (IL-6) concentrations in mice with collagen-induced arthritis, as measured by enzyme-linked immunosorbent assay. Values are the mean and SEM (n = 5 at each time point). Serum IL-6 levels in normal mice were <10 pg/ml. CII = type II collagen.

suppressed in a dose-dependent manner (Figure 2A). Suppression in the 8 mg MR16-1–treated group was statistically significant on day 35 ($P < 0.01$ versus KH-5–treated group). The mean day of arthritis onset in the 8 mg MR16-1–treated group was day 36.4 ± 2.3, and the maximum arthritis score was 1.6 ± 1.0. One of the 5 mice in this group did not develop arthritis.

In experiment 2, 8 mg of MR16-1 was administered intraperitoneally on day 0, 3, 7, 14, or 21. This treatment suppressed the development of arthritis when administered on day 0 or day 3. These suppressive effects were statistically significant at day 35 ($P < 0.001$, MR16-1 treatment on day 0 versus KH-5 treatment; $P < 0.05$, MR16-1 treatment on day 3 versus KH-5 treatment). Antiarthritic effects were not detected when MR16-1 was administered on day 7, day 14, or day 21 (Figure 2B).

Anticollagen antibody levels. In experiment 1, serum IgG anti-CII antibody levels were measured on day 34. The mean ± SEM anti-CII antibody levels in the vehicle- and KH-5–treated groups were 1,036.9 ± 73.2 units/ml (range 816.5–1,236.0) and 1,088.1 ± 89.9 units/ml (range 851.2–1,297.0), respectively. Anti-CII antibody levels were significantly lower in the 8 mg MR16-1–treated group (454.6 ± 24.3 units/ml [range 407.3–537.5]; $P < 0.01$ versus the KH-5 group). The suppressive tendency was observed for IgG2a anti-CII

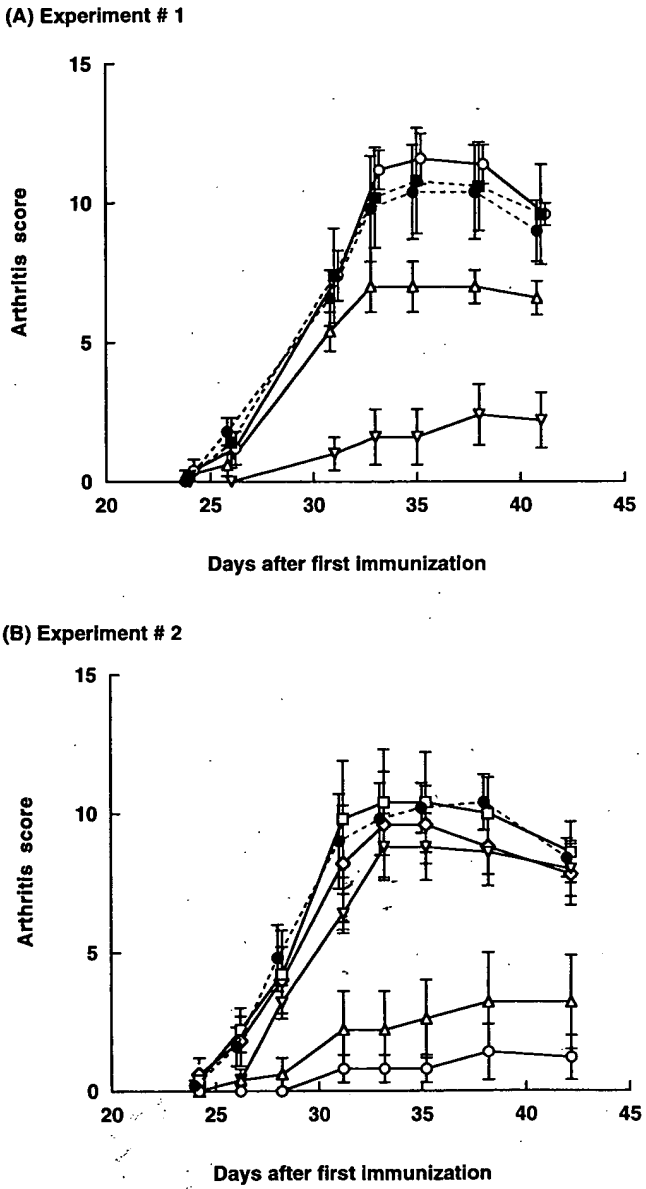

Figure 2. Antiarthritic effect of MR16-1 in mice with collagen-induced arthritis. **A,** MR16-1 or KH-5 was administered intraperitoneally on the day of the first immunization with type II collagen (CII). ● = vehicle; ■ = KH-5 (8 mg); ○ = MR16-1 (0.5 mg); △ = MR16-1 (2 mg); ▽ = MR16-1 (8 mg). **B,** MR16-1 (8 mg) or vehicle was administered intraperitoneally on the indicated day after the first immunization with CII. ● = vehicle (day 0); ○ = MR16-1 (day 0); △ = MR16-1 (day 3); ▽ = MR16-1 (day 7); □ = MR16-1 (day 14); ◇ = MR16-1 (day 21). Values are the mean ± SEM arthritis score (n = 5 in each group; see Materials and Methods for scoring system).

Table 1. Responsiveness of mouse splenic lymphocytes to type II collagen (CII) and phytohemagglutinin (PHA)*

	Tritiated thymidine uptake (cpm)			
	CII studies		PHA studies	
Treatment	Medium	CII	Medium	PHA
Vehicle	1,908 ± 188	12,976 ± 450†	696 ± 63	113,485 ± 9,107
KH-5	2,160 ± 358	10,255 ± 401	760 ± 35	112,277 ± 3,150
MR16-1	924 ± 60†	2,957 ± 243‡	399 ± 46†	106,487 ± 2,017
None	1,029 ± 21§	1,294 ± 124‡	314 ± 42‡	107,079 ± 3,720

* MR16-1 and KH-5 were injected intraperitoneally on the day of the first CII immunization. Seven days after the second CII immunization, splenic lymphocytes were cultured with heat-denatured CII for 72 hours or with PHA for 48 hours. Values are the mean ± SEM of triplicate cultures.
† $P < 0.01$ versus the KH-5 group, by parametric Dunnett's multiple comparison test.
‡ $P < 0.001$ versus the KH-5 group, by parametric Dunnett's multiple comparison test.
§ $P < 0.05$ versus the KH-5 group, by parametric Dunnett's multiple comparison test.

(KH-5 group 241.7 ± 30.8 units/ml [range 152.9–336.0]; MR16-1 group 128.3 ± 18.1 units/ml [range 65.8–179.4]) and IgG2b anti-CII (KH-5 group 1,938.1 ± 630.1 units/ml [range 928.7–4,407.2]; MR16-1 group 495.7 ± 208.5 units/ml [range 206.8–1,314.9]), but not for IgG1 anti-CII (KH-5 group 994.7 ± 183.8 units/ml [range 613.6–1,592.0]; MR16-1 group 887.6 ± 165.6 units/ml [range 378.7–1,390.8]).

Splenic lymphocyte responsiveness to CII. Splenic lymphocytes from arthritic mice were cultured with heat-denatured CII for 72 hours, and with PHA for 48 hours. As shown in Table 1, lymphocytes from the vehicle- and KH-5–treated groups showed high responsiveness to CII. In contrast, the responsiveness of lymphocytes from MR16-1–treated mice was low. Responsiveness to PHA was similar among all groups. When lymphocytes were not stimulated, ^{3}H-TdR uptake in the vehicle- and KH-5–treated groups was higher than in normal mice, and ^{3}H-TdR uptake in the MR16-1–treated group was equivalent to that in normal mice.

DISCUSSION

In the present study, we demonstrated that the administration of rat anti-murine IL-6R mAb MR16-1 suppressed the development and reduced the severity of collagen-induced arthritis. These beneficial effects were achieved when MR16-1 was administered on either day 0 or day 3. The production of IgG-class anti-CII antibody and the responsiveness of splenic lymphocytes to CII were also suppressed. The beneficial effects of MR16-1 could be induced by blocking of the IL-6R, as suggested by the findings that 1) treatment with KH-5, an isotype-matched IgG mAb, did not suppress the development of arthritis, and 2) heat-denatured MR16-1, which does not neutralize IL-6 activity, did not have any suppressive activity (data not shown).

As early as day 1, extremely high levels of IL-6 were observed in sera from mice with CIA. Since injection of antibodies or vehicle (pyrogen-free saline) into normal mice did not induce IL-6 production, this spike of IL-6 production did not appear to be due to stress or LPS contamination. Furthermore, the suppressive effect of MR16-1 on CIA development was observed only when it was administered at a very early phase (day 0 or day 3). These lines of evidence suggest that IL-6 induced immediately by CII immunization plays an important role in CIA development, and that the suppressive effect of MR16-1 may be due to the inhibition of IL-6 signal transduction in this phase.

In the 8 mg MR16-1–treated group, serum levels of IL-6 were much higher than in the vehicle- or 8 mg KH-5–treated groups on day 1 (KH-5 group 232.9 ± 24.0 pg/ml [mean ± SEM]; MR16-1 group >2,000 pg/ml). Although the exact reason for this is unclear, there are 2 possibilities. First, IL-6 could be accumulated in the blood because of the blocking of IL-6R by MR16-1. In experiments using an IL-6–dependent cell line, we found that sera from vehicle- or KH-5–treated mice had IL-6 biologic activity, but sera from MR16-1–treated mice did not. This result may be due to the existence of sufficient unbound MR16-1 to inhibit the IL-6 bioassay. In any case, this phenomenon strongly suggests that MR16-1 blocks IL-6R in vivo. In addition, although there are several reports on the antiinflammatory effects of IL-6 (8,9), accumulated IL-6 might not be related to the inhibition of arthritis. Second, MR16-1 could up-regulate IL-6 production directly. However, this is not likely since MR16-1 did not augment IL-6 production in normal mice.

On day 28, elevated production of IL-6 was detectable in mice with arthritis. The CII booster might participate in this IL-6 production. However, MR16-1 did not suppress the development of arthritis when it was administered on day 21 (just before the booster), suggesting that in the late phase, IL-6 does not play an important role in the course of arthritis. It is possible, however, that the amount of MR16-1 administered was not sufficient to suppress CIA development.

Treatment of CIA with anti-TNFα and anti–IL-1 antibodies has been shown to have an apparent suppressive effect on established arthritis (3,4). In contrast,

MR16-1 was effective only at a very early phase (prior to the development of symptoms of arthritis), as noted above. This suggests that the active phase of IL-6 is earlier in the course of CIA than that of TNFα or IL-1.

The mechanism of action of MR16-1 is not fully understood. CIA is known to be mediated by both CII-specific T cells and antibodies (10,11). Our results indicate that MR16-1 suppresses both the proliferative response of splenic lymphocytes to CII and anti-CII antibody production. Taken together with the fact that IL-6 induces T cell proliferation and differentiation (5), these findings show that MR16-1 may inhibit the clonal expansion of CII-specific T cells. It was recently reported that the T helper response shifts from the Th1 type to the Th2 type during the course of CIA (12). Since we recently found that MR16-1 suppressed the delayed-type hypersensitivity response (Mihara M et al: manuscript in preparation), the suppression of CIA by MR16-1 may result from the suppression of the Th1-type response. Therefore, the suppressive effect on IgG1 production might be weaker than on other subclasses. Further studies to examine the mechanism of action are underway.

MR16-1 binds to IL-6R on cell surfaces, as well as to soluble IL-6R. Therefore, one cannot exclude the possibility that it induces the deletion of IL-6R–positive cells. However, since MR16-1 is a rat IgG1 which does not bind to complement, the deletion of IL-6R–positive cells might not be induced by MR16-1.

It has recently been reported that IL-6 knockout mice do not develop CIA and do not produce anti-CII antibody (13). This result is consistent with our findings.

In conclusion, IL-6 is a key cytokine in the pathogenesis of CIA. The value of anti–IL-6R mAb as an effective treatment of inflammatory arthritis should be investigated further.

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Characterization of anti-mouse interleukin-6 receptor antibody☆

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Abstract

Hybridoma that produces rat anti-mouse interleukin 6 receptor (IL-6R) antibody, MR16-1, was established by the fusion of mouse P3U1 myeloma cells and spleen cells from mouse soluble IL-6R (sIL-6R)-immunized Wistar rat. In the present study, we examined the characteristics of MR16-1 in vitro and in vivo. MR16-1 bound to mouse sIL-6R dose-dependently. MR16-1 suppressed IL-6-induced proliferation of 7TD1 cells in a dose-dependent manner and this inhibitory effect was reversed by the addition of a higher concentration of IL-6. Cross-reactivity study using T cells from mouse, rat, and human revealed that MR16-1 did not cross-react with human and rat IL-6R. Binding region analysis using several human–mouse chimeric IL-6Rs showed that half of the fibronectin domain II of mouse IL-6R (amino acids 214–285) was required for MR16-1 binding. Furthermore, MR16-1 completely suppressed IL-6-induced antibody production in DNP–KLH immunized mice. These lines of evidence demonstrate that MR16-1 is useful to investigate the physiological and pathological roles of IL-6 and sIL-6R in mice.

Keywords: Mouse IL-6 receptor; Antibody; Characterization

1. Introduction

Interleukin 6 (IL-6) is a multifunctional cytokine and plays essential roles in host defense mechanisms through the immune system, hematopoiesis, and the central nervous system [1]. IL-6 exerts its biological activities through two membrane molecules, a ligand-binding 80 kDa chain (IL-6R) and a non-ligand-binding signal transducer gp130. After binding of IL-6 to membrane bound IL-6R, IL-6/IL-6R complex associates with gp130, and then the signal is transduced into the cell. Soluble IL-6R (sIL-6R), which lacks transmembrane and cytoplasmic domains, can associate with gp130 in the presence of IL-6 and transduce the signals through gp130 [2].

Elevated production of IL-6 causes many kinds of diseases including inflammatory disorders such as rheumatoid arthritis [3], mesangial proliferative glomerulonephritis [4], Crohn's disease [5], and Castleman's disease [6]. In Castleman's disease and rheumatoid arthritis, anti-human IL-6R antibody (MRA) significantly improved disease activity, suggesting that IL-6 plays essential roles in the pathogenesis of these two diseases [7,8]. To predict the clinical efficacy of anti-IL-6R antibody in IL-6-elevated diseases and to analyze the biological function of IL-6, monoclonal antibody against mouse IL-6R, which neutralizes IL-6 signal transduction, is a useful tool. However, there are only a few anti-rodent IL-6R antibodies reported at present.

In this study, we established hybridoma that produces rat anti-mouse IL-6R antibody, MR16-1, and examined the characteristics of the antibody.

2. Materials and methods

2.1. Purification of soluble mouse IL-6R

The cDNA encoding extracellular region of mouse IL-6R was constructed as described previously [9]. The

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cDNA was introduced into CHO cells to generate transformants expressing mouse sIL-6R (mSR323). mSR323 was purified from culture supernatants of CHO cells by affinity chromatography using rat monoclonal anti-mIL-6R antibody, RS12 [9].

2.2. Preparation of rat anti-mIL-6R monoclonal antibody MR16-1

Wistar rats were immunized subcutaneously with 50 μg of purified mSR323 in Freund's complete adjuvant and followed by seven boosts of 50 μg of mSR323 in the same manner. At the final boost, the rats were injected intravenously with 50 μg of mSR323 in phosphate buffered saline. After confirmation of elevated anti-mouse sIL-6R antibodies in serum, spleen cells were fused to mouse P3U1 myeloma cells with polyethylene glycol 1500 (Roche Diagnostics, Basel, Switzerland). After overnight incubation at 37 °C in wells of a 96-well plate with 100 μl of RPMI1640 medium containing 10% fetal bovine serum (FBS, Hyclone Labs, Logan, UT) and 10% BM-condimed H1 (Roche Diagnostics), 100 μl of HAT medium (RPMI1640 medium containing 10% FBS, 5% BM-condimed H1 and 2% HAT × 50 concentrate (Dainippon Pharmaceutical, Osaka, Japan)) was added to each well. On day 2, 3 and 5, half of the culture medium was replaced with fresh HAT medium. On day 7, hybridomas were screened for production of anti-sIL-6R by ELISA described below. Hybridoma clones producing anti-sIL-6R with high binding capacity were selected twice by limiting dilution. For ascites production, hybridomas (1×10^6) were injected intraperitoneally into pristine-pretreated BALB/c nude mice (Charles River Japan, Kanagawa, Japan). Ascites fluid was collected after 2 weeks, and MR16-1 was purified with Protein-G column (Pharmacia, Uppsla, Sweden).

2.3. ELISA for anti-sIL-6R antibody

Immunoplate was coated with goat anti-rat IgG polyclonal antibody (1 μg/ml, Organon Teknika, Durham, NC). After blocking with 1%BSA–PBS, serum samples were added to the wells and incubated for 60 min at room temperature. After washing, mSR323 (100 ng/ml) was added to each well and incubated for 60 min at room temperature. After washing, rabbit anti-mouse IL-6R polyclonal antibody (1 μg/ml, prepared in our laboratories) was added and incubated for 60 min at room temperature. After washing, alkaline phosphatase-conjugated goat anti-rabbit IgG polyclonal antibody (Tago, Burlingame, CA) was added to each well and incubated for 60 min at room temperature. After final washing, substrate (Sigma, St. Louis, MO) was added and the absorbance at 405 nm was measured using a plate reader.

2.4. Binding assay to mSR323

Immunoplate was coated with 10 μg/ml of mSR323 or BSA. After blocking with Block Ace (Dainippon Pharmaceutical), serially diluted MR16-1 or rat IgG (Organon Teknika) was added to each well and incubated for 60 min at room temperature. After washing, alkaline phosphatase-conjugated anti-rat IgG antibody (32 ng/ml, Biosource, Camarillo, CA) was added to each well and incubated for 60 min at room temperature. After final washing, substrate (Sigma) was added and then the absorbance at 405 nm was measured using a plate reader.

2.5. 7TD1 cell proliferation assay

Mouse B cell hybridoma, 7TD1 cell (5000 cells per well, Riken cell bank) [10] were cultured with serially diluted either MR16-1 or rat IgG (Organon Teknika) in the presence of various concentrations of recombinant mouse IL-6 (rmIL-6, R&D systems, Minneapolis, MN) for 3 days in a 96-well plate with RPMI1640 containing 5% FBS and 25 μmol/l of 2-mercaptoethanol. After culture, 10 μl of WST-8 reagent (Nacalai tesque, Kyoto, Japan) was added to each well and incubated at 37 °C for 2 h. After stopping coloring reaction by adding 10 μl of 0.1 mol/l HCl, the absorbance at 450 nm was measured using a plate reader. Relative 7TD1 growth was calculated by dividing each measured value by the average value of control wells.

2.6. T cell proliferation assay

Spleens from rats (S.D., 7–9-weeks-old, male, Charles River, Japan) and mice (BALB/cAnN, 8–10-weeks-old, male, Charles River, Japan) were pooled and dispersed to single cells. After the cells were incubated for 2 h at 37 °C in 5% CO_2, non-adhering cells were collected and T cells were obtained by passage through a nylon fiber column (Wako Pure Chemical Industries, Osaka, Japan).

Human peripheral blood mononuclear cells from healthy subjects were separated by centrifugation on Ficoll-Paque® PLUS (Amersham Pharmacia Biotech, Uppla, Sweden). The cells obtained were cultured for 2 h at 37 °C in 5% CO_2, and the adhering cells were removed. T cells were thus concentrated and purified by separating B cells from non-adhering cells using the Human T Cell Recovery Column Kit (Cedarlane Laboratories, Ontario, Canada). The cells were suspended in the RPMI1640 culture medium containing 5% FBS.

T cells at concentrations of 2×10^5 (mouse and rat) or 10^5 (human) were cultured with PHA (2 μg/ml for mouse and rat, and 0.15 μg/ml for human) (HA16, Murex BioTech, Dartford, UK), 10 ng/ml of recombi-

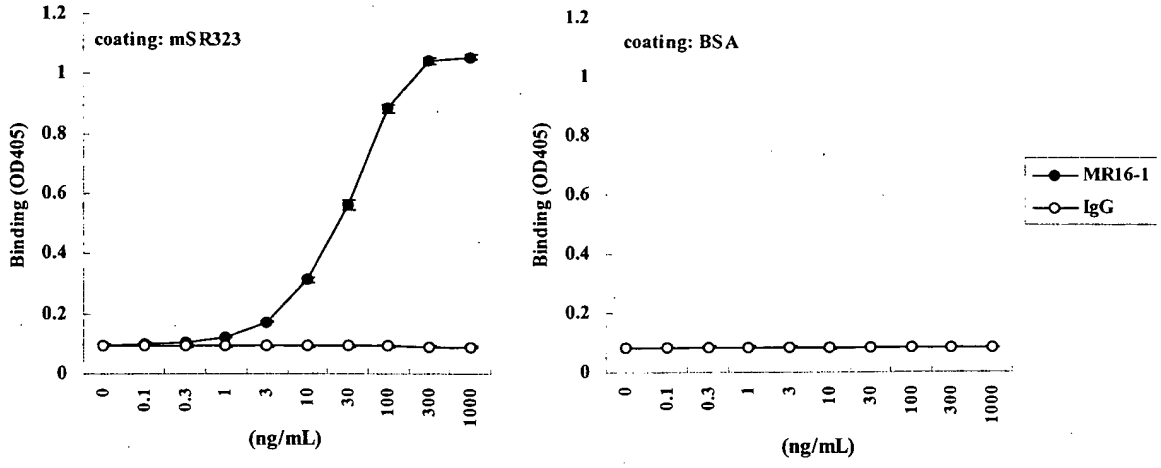


Fig. 1. Binding of MR16-1 to mouse sIL-6R. Binding activity of MR16-1 to sIL-6R was measured by ELISA as described in the Section 2. The data indicate the mean and S.D. of three wells as the absorbance at 405 nm.

nant human IL-6 (Genzyme, Cambridge, MA) and several concentrations of MR16-1 for 2 days at 37 °C in RPMI1640 containing 5% FBS in a 96-well plate. [^{3}H]-Thymidine ([^{3}H]-TdR, 1 mCi per well, Amersham, Buckinghamshire, UK) was added during the last 6 h of culture, and then [^{3}H]-TdR incorporation into the cells was measured using a liquid scintillation counter.

2.7. Construction of human–mouse chimeric IL-6Rs

Human–mouse chimeric IL-6Rs (listed in Fig. 4A) were constructed using soluble human IL-6R SR344 (#1) [1] by replacing domains consisting of the extracellular region (immunoglobulin domain, fibronectin domain I and II) with those of mouse IL-6R. Construction strategies for each chimeric receptor are briefly described as follows.

#2: Mouse sIL-6R cDNA (amino acids 1–323) was digested with *Aor*13HI and *Xho*I and the resulting cDNA fragment encoding two fibronectin domains of the extracellular region was fused to those encoding the N-terminal immunoglobulin domain of soluble human IL-6R (#2′) cloned in pcDNA3.1 (+) vector (Invitrogen, Carlsbad, CA). To obtain the cDNA fragment encoding amino acids 324–344 of mouse IL-6R, total RNA was extracted from 7TD1 cells using Isogen (Nippon Gene, Tokyo, Japan), and cDNA was synthesized from 1 μg of total RNA and random nonanucleotide primers using AMV reverse transcriptase (Takara, Osaka, Japan). Subsequent PCR amplification was performed using primers for soluble mouse IL-6R (amino acids 1–344): 5′-AACTTAAGATGCTGACCGTCGGCTGC-3′ (sense) and 5′-CCTCTAGACTAACTTTCGTACTGATC-3′ (antisense). The PCR product was digested with *Pma*CI and *Xba*I, and the resulting fragment encoding amino acids 286–344 was cloned into the corresponding region of #2′ to generate the chimeric receptor #2 (human 1–123/mouse 124–344).

#3: PCR amplification was performed using primers for fibronectin domain I of human IL-6R: 5′-GCTTCCGGAAGAGCCCCCTCA-3′ (sense) and 5′-GGCGGATCCGGCTGCAAGATT-3′ (antisense) and SR344 cDNA as a template. The PCR product was digested with *Aor*13HI and *Bam*HI, and the cDNA fragment was fused to mouse IL-6R cDNA encoding fibronectin domain II. The resulting cDNA fragment was further fused to those encoding N-terminal immunoglobulin domain of SR344 cloned in pcDNA3.1 (+) vector. The C-terminal region (*Pma*CI–*Xba*I, amino acids 286–323) was replaced with corresponding region of hmSR344 to generate the chimeric receptor #3 (human 1–217/mouse 218–344).

#4: PCR amplification was performed using primers for fibronectin domain II of human IL-6R: 5′-GCCGGATCCGCCTGCCAACAT-3′ (sense) and 5′-CCTCGAGCTAGAGAATATTATC-3′ (antisense) and SR344 cDNA as a template. The PCR product was digested with *Bam*HI and *Xho*I, and the cDNA fragment was fused to mouse IL-6R cDNA encoding fibronectin domain I. The resulting cDNA fragment was further fused to those encoding N-terminal immunoglobulin domain of SR344 cloned in pcDNA3.1 (+) vector to generate the chimeric receptor #3 (human 1–123/mouse 124–217/human 218–344).

#5: The C-terminal region (*Pma*CI–*Xba*I, amino acids 286–344) of SR344/pcDNA3.1 (+) was replaced with the corresponding region of #2 to generate the chimeric receptor #5 (human 1–285/mouse 286–344).

#6: The C-terminal region (*Pma*CI–*Xba*I, amino acids 286–344) of #3 was replaced with the corresponding region of #4 to generate the chimeric receptor #6 (human 1–217/mouse 218–285/human 286–344).

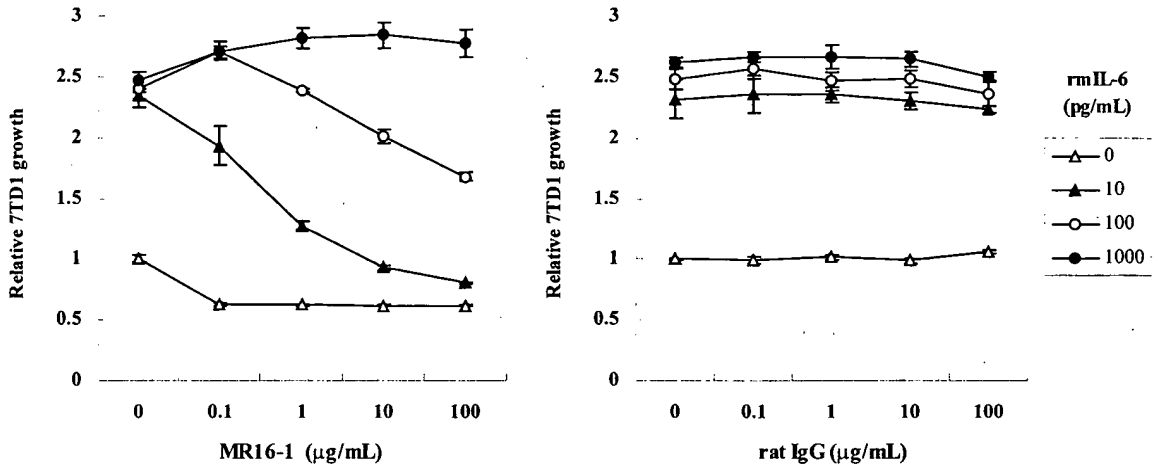


Fig. 2. Competitive inhibition of IL-6-induced 7TD1 proliferation by MR16-1. 7TD1 cells (5000 cells) were cultured with serially diluted either MR16-1 (left panel) or normal rat IgG (right panel) in the presence of various concentration of mouse IL-6. After 3 days, cell growth was measured using the WST-8 reagent. The values are represented as the ratio of those obtained with the control cells (no additives). The data indicate the mean and S.D. of three wells.

#7: The C-terminal region (*Pma*CI–*Xba*I, amino acids 286–344) of #4 was replaced with corresponding region of #2 to generate the chimeric receptor #7 (human 1–123/mouse 124–217/human 218–285/mouse 286–344).

#8: The C-terminal region (*Pma*CI–*Xba*I, amino acids 286–323) of #2′ was replaced with corresponding region of #4 to generate the chimeric receptor #8 (human 1–123/mouse 124–285/human 286–344).

2.8. Expression of human–mouse chimeric IL-6Rs

Human–mouse chimeric IL-6Rs were transiently expressed in COS-7 cells (Riken cell bank) using Trans-IT LT1 transfection reagent (Mirus, Madison, WI). After 3 days, culture supernatants of each chimeric IL-6R transfectants were collected, and expression of chimeric IL-6R proteins was confirmed by Western blotting using mouse monoclonal anti-human IL-6R antibody MT18 [7]. Briefly, 5 μl of culture supernatants were separated in a 4–20% ingredient polyacrylamide gel (Tefco, Tokyo, Japan) under the reducing condition, and transferred onto a PVDF membrane (Millipore, Bedford, MA). After blocking with Block Ace (Dainippon Pharmaceutical), the blot was incubated with 1 μg/ml of MT18 and followed by the incubation with ALP conjugated anti-mouse IgG (Zymed, San Francisco, CA). After final washing, signals were detected using BCIP/NBT (Roche Diagnostics, Mannheim, Germany) as a substrate.

2.9. Mapping of MR16-1, MRA and IL-6 binding site on IL-6R using human–mouse chimeric IL-6Rs

COS-7 culture supernatants of human–mouse chimeric IL-6Rs were incubated for 2 h in wells coated with MT18 at 5 μg/ml. Each well was incubated with 100 ng/ml of either MR16-1, MRA (humanized monoclonal anti-human IL-6R antibody; Chugai pharmaceuticals, Tokyo, Japan) or rhIL-6 for 2 h and followed by incubation with 32 ng/ml of ALP conjugated anti-rat IgG antibody (Biosource) (for MR16-1), 25 ng/ml of ALP conjugated anti-human IgG antibody (Biosource) (for MRA), or 50 ng/ml of biotinylated anti-human IL-6 antibody (R&D Systems). The wells incubated with biotinylated anti-human IL-6 antibody were further incubated with 100 ng/ml of ALP conjugated streptavidin (Pierce, Rockfold, IL). After final washing, ALP substrate (Sigma) was added as a substrate and the absorbance at 405 nm was measured using a plate reader.

2.10. Antigen specific antibody production in mice

Female C3H/HeJ mice, 8-week-old, (Clea Japan, Tokyo, Japan) were immunized intraperitoneally with 200 μg of DNP–KLH (day 0). Human recombinant IL-6 (10 μg) was injected subcutaneously for 5 consecutive days from day 1. Seven and 14 days after immunization, serum was collected and anti-DNP antibody levels were measured by ELISA. MR16-1 was injected intraperitoneally on days 0, 2, and 5.

2.11. ELISA for anti-DNP antibody

Serum levels of anti-DNP antibody were measured by ELISA [11]. Briefly, diluted mouse serum was added to the well pre-coated with DNP–BSA (100 μg/ml) for 2 h at room temperature. After washing, peroxidase-conjugated goat anti-mouse IgM (Organon Teknika), goat anti-mouse IgG (Organon Teknika), rabbit anti-mouse IgG1 (Zymed), rabbit anti-mouse IgG2a (Zymed),

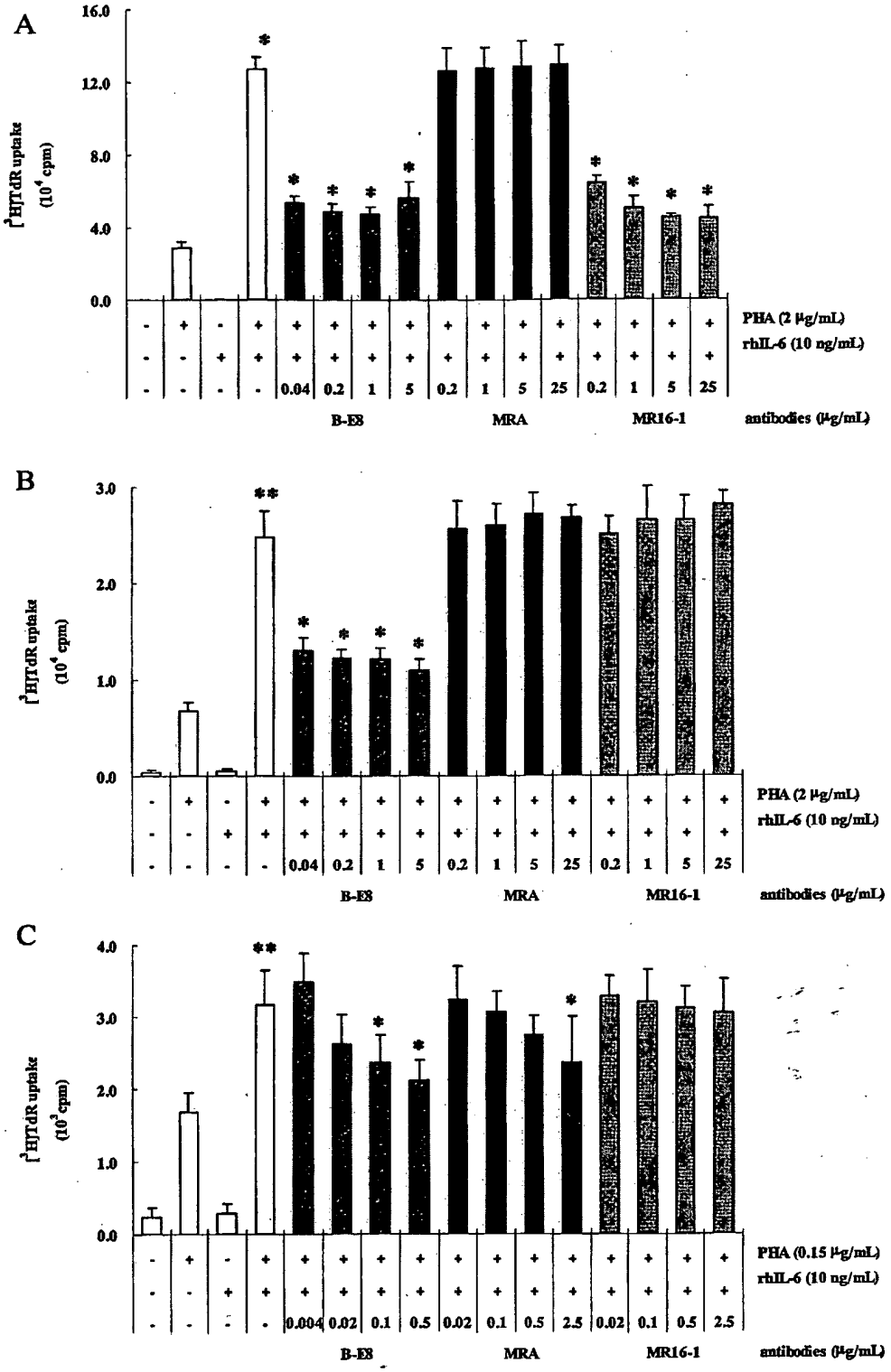
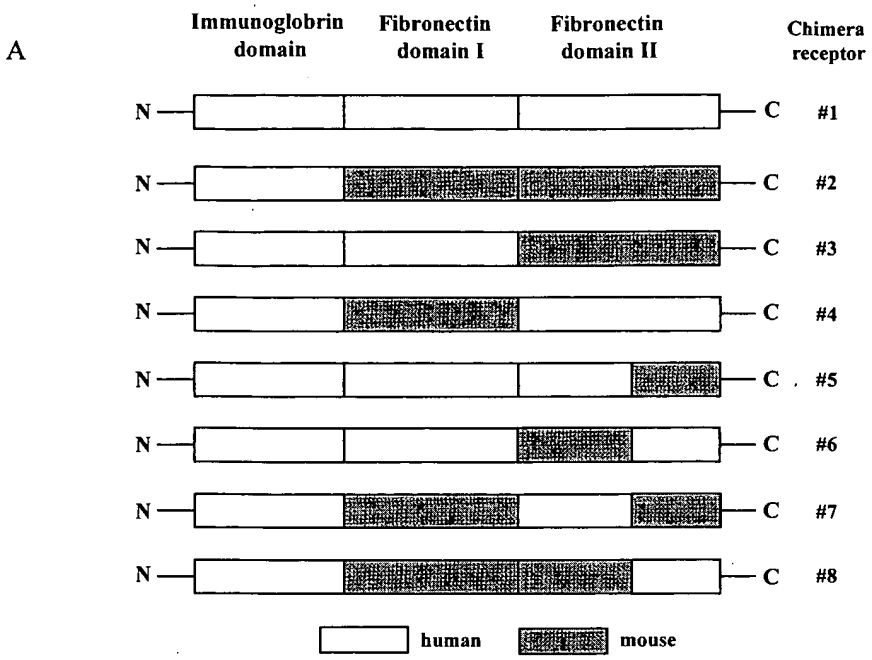
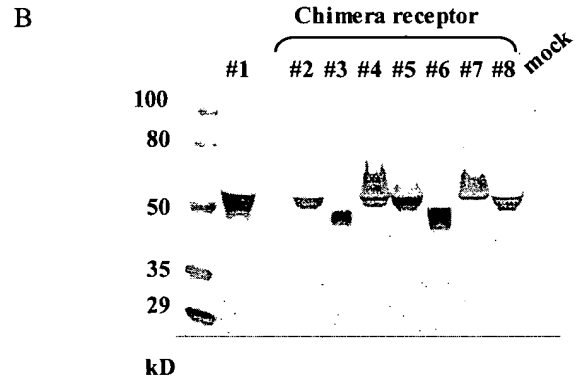


Fig. 3. Species cross-reactivity of MR16-1. T cells (2×10^5 cells for mouse and rat, 1×10^5 cells for human) were incubated with serially diluted B-E8 (anti-human IL-6 antibody), MRA (anti-human IL-6R antibody) or MR16-1 in the presence of the indicated dose of human IL-6 and PHA. Two days later, [^{3}H]-TdR uptake was measured. The data indicate the mean and S.D. of six wells. Statistical significances were analyzed by unpaired t-test and Dunnett's multiple comparison test. **, $P < 0.05$; IL-6+PHA group vs. PHA group (unpaired t-test). *, $P < 0.05$; IL-6+PHA group vs. antibody-treated group (Dunnett).





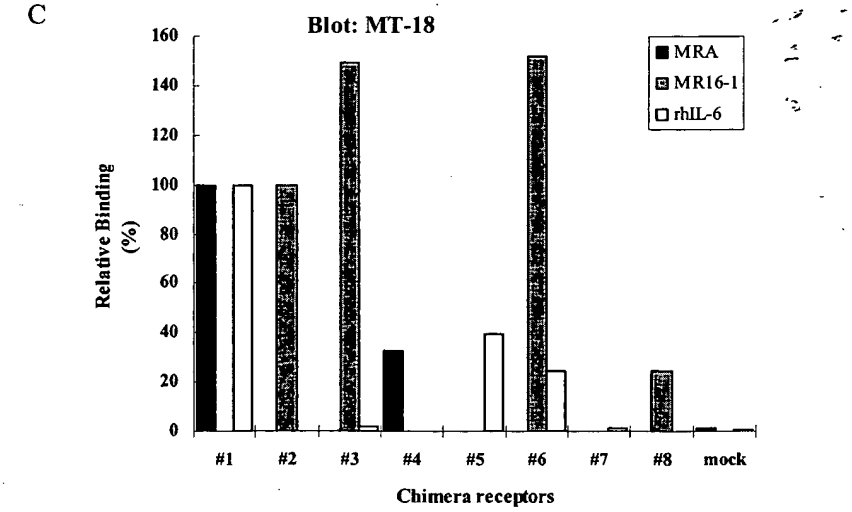


Fig. 4 (*Continued*)

rabbit anti-mouse IgG2b (Zymed), goat anti-mouse IgG3 (Nordic Immunology, Tilburg, The Netherlands) was added for 2 h at room temperature. After washing, substrate solution (0.4 mg/ml, *o*-phenylenediamine, Nacalai) was added to start the color reaction. And then the color reaction was stopped with 12 mol/l H_2SO_4 solution and the absorbance at 490–550 nm was measured with a microplate reader (Bio-Rad). Antibody content was determined from the dose response curve prepared from the pooled serum of IL-6 (−) group (day 7 for IgM, day 14 for IgGs), which was defined as 100 U/ml.

3. Results

3.1. Binding capacity of MR16-1 to antigen

The simple and specific ELISA system was established to assess binding capacity of MR16-1 to antigen using purified mSR323 directly immobilized on 96-well immunoplate. In this system, MR16-1 showed dose-dependent binding only to mSR323 (Fig. 1). Rat IgG did not bind mSR323 at all.

3.2. 7TD1 cell proliferation assay

7TD1 cell is mouse B cell hybridoma that grows in response to IL-6. As shown in Fig. 2, 10–1000 pg/ml of rmIL-6 markedly stimulates 7TD1 cell proliferation. MR16-1 dose-dependently suppressed 7TD1 cell proliferation induced by 10 and 100 pg/ml of mouse IL-6. However, the inhibitory effects of MR16-1 were reversed in the presence of a higher concentration of mouse IL-6, and no inhibition was observed in the presence of 1000 pg/ml of mIL-6.

3.3. Species cross-reactivity of MR16-1

Species cross-reactivity of MR16-1 was examined using T cells from mouse, rat and human. As shown in Fig. 3, IL-6 significantly augmented [^{3}H]-TdR incorporation in the combination of PHA compared with PHA alone in all species. Anti-human IL-6 antibody produced a suppressive effect in all species, whereas MR16-1 inhibited [^{3}H]-TdR incorporation only in mouse. On the other hand, humanized anti-human IL-6R antibody, MRA, suppressed [^{3}H]-TdR incorporation only in human.

3.4. MR16-1 binding region on mouse IL-6R

To identify MR16-1 binding region on mouse IL-6R, human–mouse chimeric IL-6Rs were constructed. Because MR16-1 did not show cross-reactivity to human IL-6R (Fig. 3), chimeric IL-6Rs in which domains are replaced between human and mouse IL-6Rs are suitable for mapping regions required for MR16-1 binding. Chimeric receptors listed in Fig. 4A were expressed in COS-7 cells, immobilized by anti-human IL-6R antibody MT18 which recognizes the immunoglobulin domain of human IL-6R, and was assessed for binding to MR16-1, MRA, and rhIL-6 by ELISA. As shown in Fig. 4B, a comparable level of chimeric receptors were detectable in culture supernatants of COS-7 cells. Among these receptors, #2, #3, #6 and #8 showed binding capacity to MR16-1 (Fig. 4B), indicating that the N-terminal half of the fibronectin domain II of mouse IL-6R (amino acids 214–285) is required for MR16-1 binding.

Anti-human IL-6R antibody (MRA) also required the fibronectin domain II of human IL-6R for binding, but further division of this domain resulted in loss of binding capacity to MRA. As MRA did not bind the reduced form of human IL-6R (data not shown), MRA may recognize the tertiary structure of fibronectin domain II of human IL-6R.

3.5. Effect of MR16-1 on IL-6-induced antibody production

Injection of IL-6 significantly augmented both IgM and IgG anti-DNP antibody production. IL-6 more strongly augmented IgG antibody production than IgM production. In addition, the production of all IgG subclasses was augmented (IgG1; 2–3-fold, IgG2a; 4-fold, IgG2b; 5–6-fold, IgG3; 6-fold). The treatment of MR16-1 significantly suppressed antigen specific antibody production (Fig. 5). However, the degree of suppression did not cross IL-6 non-treated levels. When we examined the inhibitory effect on antigen specific antibody production without IL-6 injection, MR16-1 did not show any suppressive effect (data not shown).

Fig. 4. Binding of human–mouse chimeric IL-6Rs to MRA, MR16-1 and IL-6. (A) Schematic representation of human–mouse chimeric IL-6Rs. (B) Western blotting analysis of chimeric IL-6Rs expressed in COS-7 cells. Total cell lysates of COS-7 cells transfected with each chimeric IL-6R were separated on SDS polyacrylamide gel and transferred to a PVDF membrane. The blot was probed with MT18 and followed by the incubation with ALP conjugated anti-mouse IgG. Signals were detected using BCIP/NBT as a substrate. (C) Binding of chimeric IL-6Rs to MRA, MR16-1 and rhIL-6. Binding capacity of each chimeric IL-6R was assessed by ELISA as described in the Section 2. Data indicate the mean of two wells. The values are presented as the percentage of those obtained with wild type sIL-6R (#1) (for MRA and rhIL-6 binding) or with #2 (for MR16-1 binding).

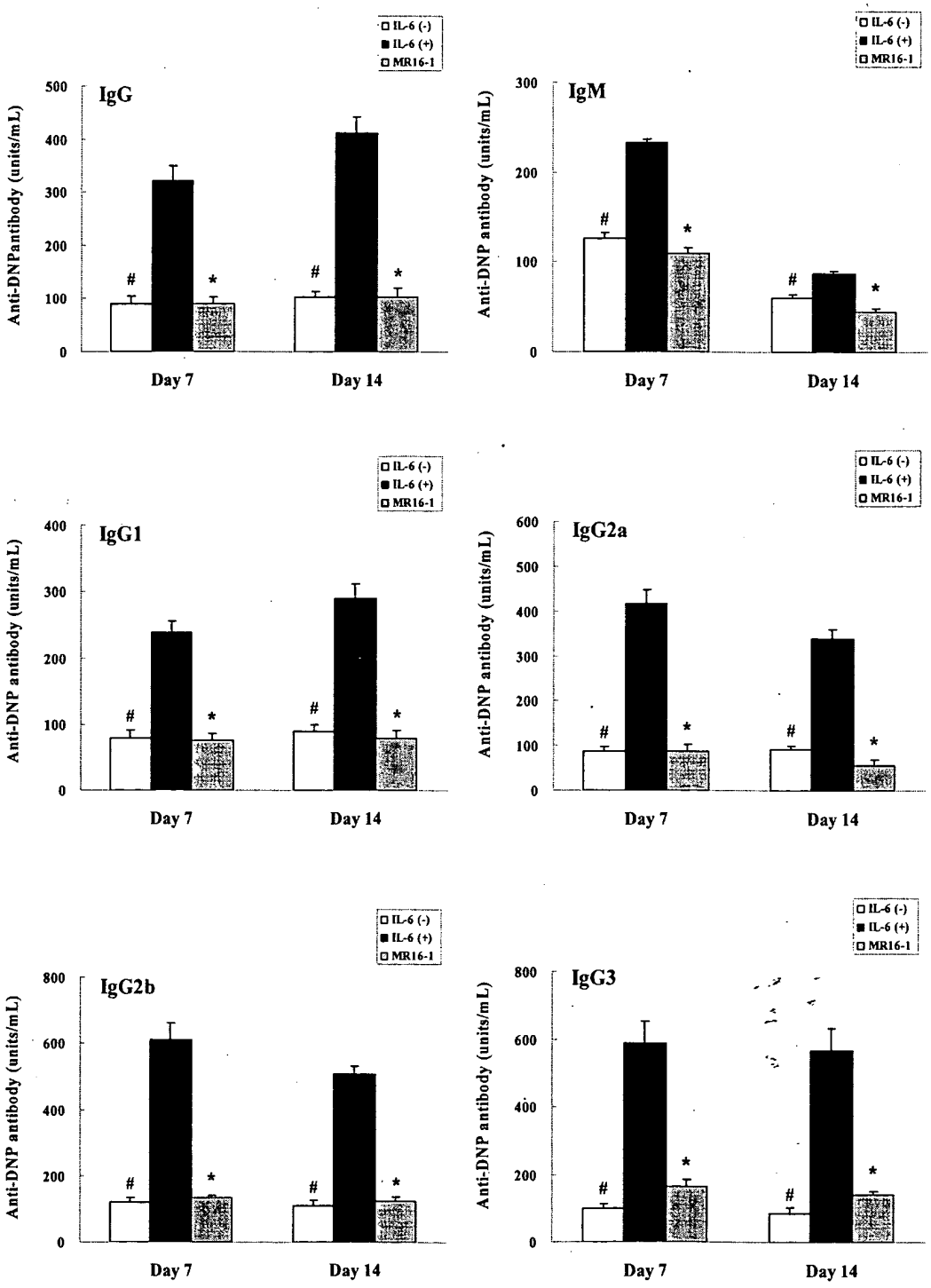


Fig. 5. Suppressive effect of MR16-1 on antibody production in mice. Female C3H/HeJ mice were immunized i.p. with DNP–KLH in saline on day 0 and were bled on days 7 and 14. Human IL-6 (10 μg) was injected subcutaneously for 5 consecutive days from day 1. MR16-1 was injected i.p. on days 0, 2 and 5. Seven and 14 days after immunization, serum was collected and anti-DNP antibody levels were measured by ELISA. Data indicate the mean and S.E. of five mice. Statistical significances were analyzed by unpaired t test. *, $P < 0.05$; IL-6 (+) vs. MR16-1, #, $P < 0.05$, IL-6 (−) vs. IL-6 (+).

4. Discussion

In the present study, we generated monoclonal antibody to mouse IL-6R and examined its characteristics in vitro and in vivo.

MR16-1 suppressed IL-6-induced proliferation of IL-6-dependent cell line, 7TD1 cells dose-dependently. However, this suppressive effect was not observed, when high-dose IL-6 was used. This experiment indicates that MR16-1 is a neutralizing antibody and

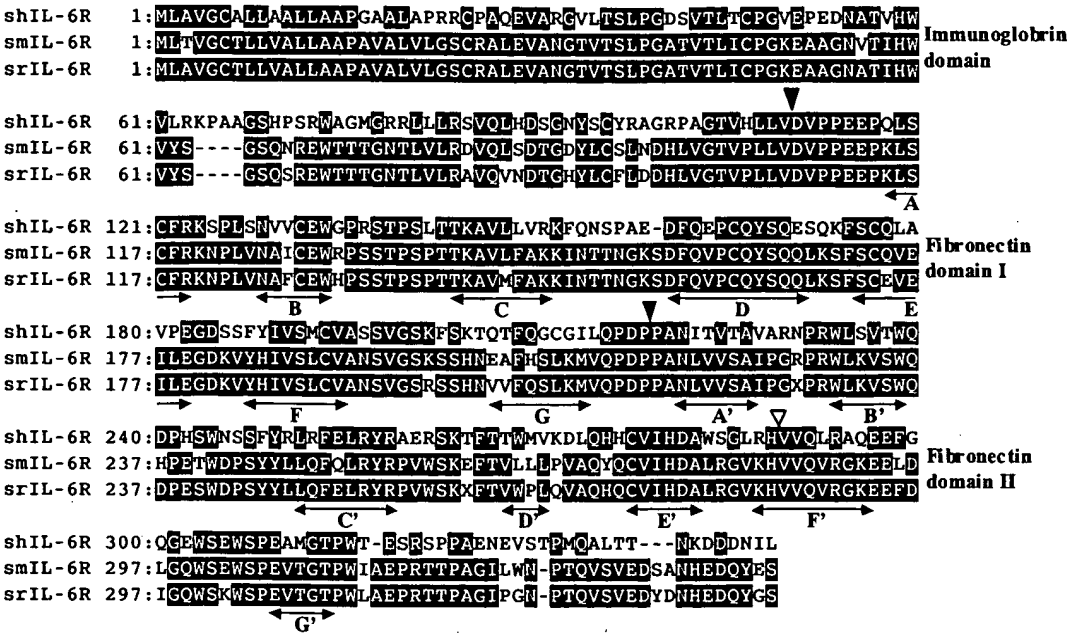


Fig. 6. Amino acid sequence alignment of human, mouse and rat IL-6R. Amino acid sequences of the extracellular region (amino acid 1–344) of human, mouse and rat IL-6Rs were aligned by GENETYX SV/RC ver. 5.0.4. Amino acids conserved in more than two species shown in inverted letters. The junctions of each domain are indicated by closed arrowheads. The divided point in the fibronectin domain II is shown by open arrowhead. Amino acids comprising β-sheet structures are specified by arrows. Amino acid sequences of each IL-6R were obtained from Genbank accession number P08887 (human), NP 034689 (mouse), and P22273 (rat).

competitively inhibits the binding of IL-6 to IL-6R. Moreover, the administration of MR16-1 completely suppressed IL-6-induced antigen-specific antibody production in mice, indicated that MR16-1 was also neutralizing in vivo.

There are two types of IL-6R, soluble type and membrane-bound type and both types of receptor can bind to IL-6 and are involved in IL-6 signaling [2]. MR16-1 bound to sIL-6R in a dose-dependent fashion (Fig. 1). Furthermore, the addition of mSR323 promoted the proliferation of 7TD1 cells, and MR16-1 inhibited mSR323-induced proliferation (data not shown). On the other hand, although we did not check the binding of MR16-1 to membrane bound IL-6R directly, MR16-1 suppressed the proliferation of 7TD1 cells and T cells. These results suggested that MR16-1 could bind to both types of IL-6R and suppress the signal transduction of IL-6.

Cross-reactivity was defined as an inhibitory activity of T cell proliferation assay. IL-6 is reported to augment the proliferation of T cells stimulated with mitogen [12]. MR16-1 suppressed IL-6-induced proliferation of T cell from mouse, but not from rat and human, indicating that MR16-1 cannot recognize rat and human IL-6R. On the other hand, anti-human IL-6R antibody (MRA) suppressed IL-6-induced proliferation of T cell from human, but not from mouse and rat. Amino acid sequence alignment revealed that the sequence homology between mouse and human or rat IL-6R is 52 and 90%, respectively. MR16-1 probably recognizes regions that are not conserved in human and rat IL-6R.

Studies using human–mouse chimeric receptors further provided evidence that MR16-1 specifically binds to mouse IL-6R at the N-terminal half of fibronectin domain II. This region contains the B′–C′ loop of fibronectin domain II, the predicted IL-6 binding site on human IL-6R [13,14]. In the B′–C′ loop of mouse IL-6R, there are two amino acids, H237 and T240, that are not conserved in human and rat sequences (Fig. 6). However, the mouse sIL-6 R in which the B′–C′ loop was replaced with human B′–C′ loop, retained binding capacity to MR16-1, and the human sIL-6R in which the B′–C′ loop was replaced with mouse B′–C′ loop failed to bind MR16-1 (data not shown), suggesting that H237 and T240 are not involved in MR16-1 binding. L265-Y272 in D′ β-strand and D′–E′ loop is another possible epitope of MR16-1 in the N-terminal half of fibronectin domain II, because this sequence is also unique to mouse IL-6R. Although L265-Y272 is not included in the binding surface with IL-6 (B′–C′ loop and F′–G′ loop), this region is adjacent to both B′–C′ loop and E′ β-strand, the binding surface with IL-6 and gp130, respectively [15]. Moreover, inhibitory effects of MR16-1 on IL-6 induced 7TD1 cell proliferation was reversed by a higher dose of IL-6, suggesting that MR16-1 competitively inhibits IL-6 activities by interfering with the access of IL-6 to its binding site or the assembly of the IL-6/IL-6R/gp130 complex. On the other hand, MRA requires both the N-

terminal and C-terminal half of the fibronectin domain II. Thus MRA is likely to bind just to the binding pocket with IL-6 consisting of B′–C′ loop and F′–G′ loop. Although the epitope of MR16-1 and MRA may be different, the mode of IL-6 inhibitory action of MR16-1 is similar to that of MRA. Further studies such as X-ray crystallography are needed for precise definition of the MR16-1 binding epitope.

In conclusion, MR16-1 is specific antibody to mouse IL-6R with a strong neutralizing activity. IL-6 is thought to play an important role in inflammatory diseases and certain cancers. Therefore, MR16-1 may be a valuable tool for the analysis of pathogenic roles of IL-6.

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